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THE CONTROL OF TICK INFESTATION AND A RELATED DISEASE OF LAMBS IN NORTH WALES.

BY C. L. WALTON, PH.D., M.SC.

(*Adviser in Agricultural Zoology, Department of Agriculture, University College of North Wales, Bangor.*)

IN April, 1923, the Agricultural Department of the University College of North Wales, Bangor, received complaints from sheep farmers in the Pentre Voelas district of Denbighshire, stating that many lambs were lost each year owing to the presence of a disease that is associated, apparently, with tick infestation. Specimens of ticks collected on some of the affected farms were found to be *Ixodes ricinus*.

Subsequent inquiry showed that the seriously affected area was limited in extent, and that the heaviest losses had occurred on two contiguous farms where tick infestation was severe. It should be mentioned, however, that on other farms in the vicinity many ticks may be found on the sheep and tick infestation is not uncommon in some other similar districts in North Wales, but the presence of the above-mentioned disease is not observed.

The two sheep farms most seriously affected are situated on a hill side, facing N.W. The land rises from a marshy valley, 800–850 feet above sea level, to the summit of a ridge with an elevation of about 1100 feet. The land is bare and treeless and the rainfall is heavy. The mean annual rainfall on the Alwyn catchment area on the other side of the valley is 50 inches. The soil, peaty in the valley, is loam, overlying a clay subsoil, on the slopes. Cattle are kept, and some ploughing is done on the lower enclosed land where the sheep are wintered. About the first week in May, the sheep, but not the cattle, are sent up to the hill pastures. Tick infestation usually commences as soon as these Welsh Mountain sheep, with their young lambs, go up to the hill. The two flocks (208 ewes and 100 lambs in all, in 1924) under observation run together on the same pasture. Subsequently, a third flock, from adjoining and similarly tick-infested pastures, was included in the experiments and the total number of sheep then amounted to 266 ewes and 164 lambs.

The area grazed by these flocks amounts to 119 acres and, as previously stated, has been stocked at different times with from 300 to 450 sheep approximately.

Several other farms situated on the S.E. side of the hill are stated to be slightly tick-infested, but no data are available for these. It is interesting to note, however, that adjacent farms on either side of the infested area in question are said to be free from ticks, a statement that is practically borne

out by an examination made of a flock of sheep running on one of these farms, which resulted in the finding of only one tick.

The owner of the largest flock had occupied the land for 26 years, and experienced trouble within the first year of occupation. The infestation and diseased condition continued to appear each year, varying in severity but showing a decided increase. In 1922 the trouble was present in alarming proportion.

Prior to 1922 few deaths occurred, though many lambs were affected by sores, "blisters," and swellings of the leg joints. Only lambs were obviously affected, but the general condition of the whole flock was lowered. In 1922, 13 lambs died, and of the remainder, all were so severely affected that the majority still showed the effects when adult, and it was this serious deterioration of the flock that induced the owner to seek advice from the Agricultural Department of the University College, Bangor. In 1923 there were no deaths but 30 lambs were severely affected.

On my advice an attempt was made to burn over the hill in the spring of 1924, but repeated attempts failed to accomplish anything owing to the continuous wetness of the ground.

It was then decided to institute a series of experimental dippings with the object of eradicating or at least reducing the tick infestation. Dippings at frequent but irregular intervals with various proprietary dips had proved to be useless in controlling the tick infestation, when the writer was fortunate in obtaining the co-operation of the staff of the Cooper Technical Bureau, London (particularly Dr L. E. Robinson and Mr W. C. Prudames, M.R.C.V.S.), who had previous experience in tick eradication experiments on infested sheep farms in Great Britain. By this co-operation it was possible to obtain from the Cooper Technical Bureau supplies of a special short-interval arsenical dip which could be used repeatedly at a tick-killing strength at an interval so short as five days.

The writer has also received considerable assistance from Mr R. F. Montgomerie, B.Sc., F.R.C.V.S., Adviser in Veterinary Science, and Mr R. A. Roberts, B.Sc., Lecturer in Agricultural Botany, at Bangor. Mr Montgomerie supplies the following note on the diseased condition. "The disease appears essentially to be a pyaemia caused by a mixed infection the organisms having a predilection for sites in the regions of joints. Only lambs are infected and the first symptoms may appear eight or nine days after the flocks have been turned to the hill pasture. Abscess formation in the region of one or more joints causes severe lameness. The abscess does not tend to point but develops to a cold abscess. Death may occur in the more acute stages of the disease but lambs which survive that stage may have recovered by the late autumn. The study of the bacteria present in the pus from the abscesses has not yet been completed." Mr Roberts has made a botanical examination of the pastures concerned and a copy of his report is given in an Appendix to this paper (p. 271).

Thanks are also due to the farmers whose flocks were the subject of these observations, Mr D. R. Jones, Parc; and Mr R. Jones, Gowld, nr Pentre Voelas, Denbighshire. They have throughout rendered every possible assistance in a most efficient manner.

EXPERIMENTAL DIPPINGS IN 1924.

Before being turned out on the hill pasture on 1. v. 24, the flocks had been dipped once in a proprietary dip.

On 15. v. 24, I carefully examined six sheep from the flock immediately after their collection for the first experimental dipping, with results recorded in Table I. The flock had been on the infested land for 14 days.

Table I.

	Head and neck	Under legs	Rest of body	Total ticks present
1. Ewe lamb, 7 weeks old	48	65	2	115
2. Ram lamb, 8 „	28	51	2	81
3. Ewe	95	16	12	123
4. Ewe	76	83	7	166
5. Two lambs, 2 days old	—	—	—	—

Average No. of ticks per sheep 121.

Some ewes were even more heavily infested, but these results may be taken as the average and the owners stated that this was in their opinion a usual infestation, but "not as heavy as in some years." At this date no lambs showed any adverse symptoms other than some small sores on the skin under the legs, where a number of ticks were feeding close together. Very few keds (*Melophagus ovinus*) were present, probably due to the frequent dippings these flocks had undergone in attempts to check the tick infestation. The first experimental dipping took place on this date, 15. v. 24, under the direction of Mr Prudames, using the special dip previously mentioned, the dip, in the bath, containing arsenical substances equivalent to 0·2 per cent. of As_2O_3.

It had been decided after consultation with the Cooper Bureau to try (*a*) to clear the flock by dipping, and (*b*) to eradicate ticks from the land by means of a series of dippings at five-day intervals.

On 23. v. 24 a second tick examination was made, with the results recorded in Table II.

Table II.

	Ticks
1. Ewe lamb, once dipped	19
2. Ram lamb „	14
3. Ewe „	7
4. Ewe „	1
5. Ewe lamb, twice dipped	12
6. Ram lamb „	11
7. Ewe „	0
8. Ewe „	5
Total	69

Average No. of ticks per sheep 8·6.

Numerous dead ticks found in the fleeces.

This enumeration was made on the open hill in pouring rain, and the ticks were not classified according to stage, sex, etc., and the above count refers to female ticks in all stages of engorgement found attached to the sheep.

On 6. vi. 24, the enumerations recorded in Table III were made by Dr Robinson (after four dippings).

Table III.

	No. of times dipped	No. of ticks found on sheep					
		♂♂	Fed ♀♀	Unfed ♀♀	Nymphs	Larvae	Total
1. Ewe	3	1	1	7	1 (engorged)	—	10
2. „	4	3	9	8	66 (21 on nose + 40 lower lip)	5	91
3. Lamb	3	6	—	6	3	1	16
4. „	4	9	7	9	7	1	33
5. „	3	4	—	2	7	—	13
6. „	—	—	1	—	4	—	5

Average No. of ticks per sheep 28.

Many dead ticks, ♀♀ and ♂♂, were in the fleeces. Some pairs were found *in copula*. Several keds were present, living and dead. Lambs *5* and *6* were suffering from swollen joints, and were dull and listless. These were the only members of the flock to show any of the usual symptoms during 1924.

On 11. vi. 24, the sixth dipping was carried out and a number of ewes and lambs were examined by Mr Montgomerie, who found very few ticks indeed, chiefly partially fed nymphs around the muzzle.

On 5. vii. 24, I examined 6 ewes which yielded in all 12 ticks (2 larvae, 1 ♂, 9 ♀♀) and 4 lambs which yielded 9 dead ♀♀. Average for all 10, 2·1. All the lambs were well and strong. The ewes were in rather poor condition, probably owing to the very cold wet weather. The wool also was in poor condition.

It may be mentioned that on 15. v. 24 an ordinary sweeping net was used on the mountain pasture 11.30–12 noon, in bright sunlight. From 7–10 square yards were swept at each test, and 23 such trials were made at different points. In all 20 unfed ticks were obtained, chiefly from the lee side of tufts of grass and rushes on the drier land. Unfortunately, during subsequent visits very adverse weather conditions made any further attempts impossible.

Experimental Dippings in 1925.

The experimental dippings were continued during 1925. Owing to the possibility of injury to the wool, it was decided to dip the lambs only, until after shearing time, when the whole flock would be included. Dipping, therefore, commenced on May 19th, when 114 lambs were treated. Further dippings (of lambs only) were on May 25th and 30th, and June 5th and 10th. Shearing took place on June 27th. No further dippings could be given owing to scarcity of water. The ewes, therefore, were not dipped at all in 1925.

Tick counts were made on May 8th and 25th and June 10th (this last by Dr Robinson). Figures are appended in Tables IV–VI.

On this occasion three lambs were showing symptoms of lameness, etc., the only ones during the season to be in any way affected. Two of these cases subsequently developed symptoms of the disease known as *Din-dro* or "Sway-back" (which occurs here and there in North Wales).

It will be seen from Table IV that the average number of ticks on May 8th, before any dippings had taken place, was 10·16 for 12 sheep examined. These fall into two groups: (*a*) four lambs and two ewes that had been on the hill six days, averaging 6 ticks each; (*b*) four barren ewes, one ram and one yearling, which had been on the hill 11 days, averaging 14·33 ticks each. On May 25th, just prior to the first dipping, 10 sheep averaged 19·5, namely six lambs 20·5, and four ewes 18·0.

On June 6th, 20 sheep gave an average count of 8·9; that is, 10 ewes (undipped) 12·1 and 10 lambs (four times dipped) 5·7.

Table IV. *1st tick count—Parc and Gowld*, 8. v. 1925.

	No. of ticks found on sheep					
	♂♂	Fed ♀♀	Unfed ♀♀	Nymphs	Larvae	Total
1. Ewe	2	—	—	—	—	2
2. „	2	1	7	—	—	10
3. Barren Ewe	1	3	6	5	2	17
4. „	—	6	4	2	—	12
5. Lamb	2	4	—	—	—	6
6. „	—	8	2	—	—	10
7. „	—	—	—	—	—	—
8. „	—	1	6	—	1	8
9. Yearling	—	—	6	—	2	8
10. Barren Ewe	—	—	13	1	2	16
11. „	1	—	17	—	2	20
12. Ram	2	—	8	—	2	12

Average No. of ticks per sheep 10·16.

About 100 rams, yearlings and barren ewes sent to hill on 27. iv. 25; ewes and lambs sent to hill on 2. v. 25.

Table V. *2nd tick count—Parc and Gowld*, 25. v. 1925.

	No. of ticks found on sheep					
	♂♂	Fed ♀♀	Unfed ♀♀	Nymphs	Larvae	Total
1. Lamb	—	—	2	3	28	33
2. „	6	1	5	2	—	14
3. „	1	3	—	1	1	6
4. „	1	—	2	1	7	11
5. „	—	2	1	2	5	10
6. „	1	—	—	9	39	49
7. Ewe	2	3	15	4	4	28
8. „	5	4	7	1	4	21
9. „	1	3	5	2	1	12
10. „	—	—	2	1	8	11

Average No. of ticks per sheep 19·5

„ „ on the 6 lambs 20·5

„ „ „ 4 ewes 18·0

125 lambs were dipped during fine warm weather, followed by light showers in the afternoon.

A very considerable number of the larvae and nymphs were engorged and several of the latter dropped whilst the sheep were being examined.

Table VI. *3rd tick count—Parc and Gowld*, 10. vi. 1925.

		No. of ticks found on sheep					
		♂♂	Fed ♀♀	Unfed ♀♀	Nymphs	Larvae	Total
1.	Lamb	—	—	—	—	1	1
2.	,,	—	—	—	—	4	4
3.	,,	—	—	—	—	—	0
4.	,,	—	—	4	—	5	9
5.	,,	—	—	—	—	23	23
6.	,,	—	—	—	—	4	4
7.	,,	—	—	—	—	2	2
8.	,,	—	—	—	—	6	6
9.	,,	—	—	—	—	1	1
10.	,,	—	—	—	—	7	7
11.	Ewe	—	—	1	—	1	2
12.	,,	1	—	2	—	1	4
13.	,,	—	—	—	1	8	9
14.	,,	—	8	1	—	—	9
15.	,,	—	1	—	—	18	19
16.	,,	—	—	1	1	6	8
17.	,,	1	—	5	1	10	17
18.	,,	—	—	3	—	12	15
19.	,,	1	—	5	1	10	17
20.	,,	—	—	1	—	20	21
	Average No. of ticks per sheep					8·9	
	,, ,, ,, on the 10 ewes (undipped)					12·1	
	,, ,, ,, 10 lambs (dipped)					5·7	

Experimental Dippings in 1926.

Owing to scarcity of grass, the barren ewes, rams, wethers, etc., were sent to the hill on the unusually early date of April 7th, the ewes and lambs following on May 1st (and some a little later), the whole of the flock being on the hill by 9. v. 26, amounting in all to about 290 sheep and lambs.

The entire flock was dipped on May 11th (the owners noting a number of ticks). Lambs only were dipped on May 19th, 28th and June 12th. Shearing took place on June 26th and the entire flock was again dipped on July 3rd.

Three cases of adverse symptoms were noted among the lambs (one recovered and the other two died). All showed the characteristic sore patches, knee swellings, lameness, etc. The wool condition was excellent. No cases of "Sway-back" were noted.

In 1926 the writer visited the farms on May 7th and made a tick count on 15 animals as recorded in Table VII. Some of the ewes and lambs had been on the hill 7 days, and others 14 days.

It will be seen, on comparing Tables IV and VII, that there is an increase in numbers over the first count in 1925; this may be explainable in view of the incomplete dippings of the previous summer, already mentioned.

The results of the 1926 dippings are reflected in the counts recorded in Table VIII made by Dr Robinson on July 12th, after the lambs had been dipped five times and the ewes twice.

Table VII. *Tick count—Parc and Gowld*, 7. v. 1926.

		No. of ticks found on sheep				
		♂♂	♀♀	Nymphs	Larvae	Total
1.	Lamb	—	14	—	9	23
2.	„	—	15	—	20	35
3.	„	2	11	—	26	39
4.	„	—	6	—	14	20
5.	„	—	9	—	13	22
6.	„	—	5	—	14	19
7.	„	2	8	—	19	29
8.	„	—	5	—	6	11
9.	„	—	6	—	1	7
10.	„	—	9	—	9	18
11.	Ewe	1	12	—	11	24
12.	„	2	18	—	4	24
13.	„	—	3	—	10	13
14.	„	—	8	—	20	28
15.	„	2	15	—	12	29

Average No. of ticks per sheep (omitting ♂♂) 15·46.
Numerous dead ticks found in fleeces.

Table VIII. *Result of tick count on dipped sheep—Parc and Gowld*, 12. vii. 1926.

		No. of ticks found on sheep				
		♂♂	♀♀	Nymphs	Larvae	Total
1.	Lamb	—	—	—	1	1
2.	„	—	1	—	—	1
3.	„	1	1	—	—	2
4.	„	—	—	—	2	2
5.	Ram	—	2	—	—	2
6.	Ewe	—	1	—	—	1
7.	„	—	1	—	—	1
8.	„	—	1	—	—	1
9.	„	—	1	—	—	1
10.	„	—	1	—	—	1
11.	„	—	1	—	—	1
12.	„	—	1	—	—	1
13.	„	—	1	—	—	1
14.	„	—	3	—	—	3
15.	„	—	2	—	—	2
16.	„	—	1	—	—	1
17.	„	—	1	—	—	1
18.	„	—	4	—	—	4
19.	„	—	2	—	—	2

Lambs and ewes free from tick infestation 11.
Average No. of ticks per sheep 0·96.

APPENDIX.

REPORT BY R. ALUN ROBERTS, B.Sc., UPON THE SHEEP RUN AT "GOWLD" AND "PARC," PENTRE VOELAS. (Visited in June 1924.)

The sheep walk, which has a N.N.W. aspect, with a slope varying from very slight to pronounced, represents to my mind a very variable transitory type. The flora I would regard as essentially "grass heath," at least in origin, with a "grass moor" population advancing into it. That there is a skeleton of heath flora is chiefly evinced by the presence of such plants as:

Festuca ovina	Sheep's Fescue
Deschampsia flexuosa	Mountain Hair Grass
Anthoxanthum odoratum	Sweet Vernal
Polygala vulgaris	Milkwort
Potentilla erecta	Tormentil
Vaccinium myrtillus	Bilberry
Galium saxatile	Mountain Bed Straw
Luzula campestris	Field Wood Rush
and the moss *Polytrichum*	

It is significant that only a very small percentage of the herbage is composed of *Agrostis vulgaris*—Bent Grass, which with *Festuca ovina*—Sheep's Fescue normally constitutes the bulk of the herbage on these grass heaths.

Into this skeleton "grass heath" element is firmly established as a dominant phase characteristic "grass moor" elements such as:

Eriophorum vaginatum	Cotton Grass
Nardus stricta	Mat Grass
Juncus squarrosus	Heath Rush
and *Sphagnum*	Bog Moss

These are plants which either make for peat formation (*Eriophorum* and *Sphagnum*) on lands of high rainfall or else they are favoured by wetness, either of precipitations or bad drainage (*Juncus squarrosus* and *Nardus stricta*), and by them heath may pass into "grass moor."

I would say then that the ground at Gowld and Parc represents a transition between "grass heath" and "grass moor." The high organic content of the soil points to conditions that will eventually favour the establishment of the latter. The mineral content, however, points to the continuing of the "grass heath" flora on the less peaty and drier parts. I do not anticipate a further retrogression to a *Calluna* moor (the type so common on Hiraethog adjoining, where one gets much thinner pure peat).

In conclusion I would advance two possible explanations of the more or less anomalous instability.

(1) Insufficient drainage for a continuous long period has turned the scale in favour of a "grass moor" which has not yet completely dominated the "grass heath" flora of former days.

(2) The two phases will continue to alternate—the "grass heath" being induced by periodic droughts and the "grass moor" by excessive wet. Conditions for the last two or three years may have favoured the latter so that at the time we visited the ground the balance may have been excessively in favour of the "grass moor."

In the interests of the farmers concerned, the "grass heath" is, of course, the more desirable phase, and they could induce its development (whichever of the two alternatives (1) and (2) above is operative) by draining and by periodic surface burning. If either type were permanently established to the exclusion of the other, it is outside my sphere to express an opinion as to

which scrub flora would be the less likely to foster the development of ticks. To the grazier the "grass heath" is far superior.

SUMMARY AND CONCLUSIONS.

(1) An account is given of a serious infestation in sheep and lambs by *Ixodes ricinus* in the uplands of Denbighshire, North Wales.

(2) This infestation was accompanied by serious disturbance of health resulting in the general deterioration of the flock and the death of lambs.

(3) Experimental dippings were commenced in 1924, using a special "short-interval" arsenical dip supplied by the Cooper Technical Bureau, London. Serial dippings were made each spring at five-day intervals. When possible an early autumn series was conducted.

(4) The objects were (*a*) to control the infestation, (*b*) to control the diseased condition, and (*c*) to attempt to eradicate ticks from the land.

(5) The control of both the ticks and the disease were easily effected. A few sporadic cases of disease did occur during the course of the experiment.

(6) Eradication has not yet been accomplished, but the average number of ticks was reduced from 121 per animal in May 1924 to 0·96 per animal in July 1926.

(7) It is therefore suggested that short-interval dipping for serious tick infestation in sheep is practicable on British hill farms, and that resultant mortality from disease can thereby be reduced.

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CONTRIBUTIONS TO THE ANATOMY OF SOME REPTILIAN CESTODES.

By JEAN G. BAER, D.Sc.

Zoological Institute, University of Neuchâtel.

(With 12 Text-figures.)

Having at our disposal material from various sources obtained from the intestines of monitor lizards, it appeared useful to prepare drawings and descriptions of the less known species of parasites.

(*a*) **Duthiersia fimbriata** (Diesing, 1854).

Most of our material consists of numerous specimens of *Duthiersia fimbriata* (Diesing, 1854), a Bothriocephalid, frequently found in monitors. As this species was obtained both from African and from Indian monitors, a comparative study of the various specimens was made in order to try and clear up the question as to the existence of two distinct species.

Before dealing with anatomical details, the history of the genus *Duthiersia* may be briefly recapitulated.

Valenciennes (1849) described under the name of *Bothridium du varan du Nil* a tapeworm from *Varanus niloticus* L. This species was later listed by Diesing as *Solenophorus fimbriatus* Diesing *spec. inq.* Perrier (1873) rediscovered the tapeworms from African and from Indian monitors, and not knowing of Diesing's paper, he created a new genus *Duthiersia* containing two species, viz. *D. expansa* Perrier from Indian monitors and *D. elegans* Perrier from African monitors. The genus *Duthiersia* was fully justified and was accepted by Monticelli and Crety (1891) who placed it in their new sub-family *Solenophorinae* where it figured with only a single species, *D. fimbriatus* (Diesing). The Italian authors considered *D. elegans* as a synonym of *D. expansa*, the latter being already a synonym of *D. fimbriata*. The sub-family *Solenophorinae* has since disappeared, thanks chiefly to the work of Lühe (1899), and all authors agree with Lühe in placing the genus *Duthiersia* in the sub-family *Diphyllobothriinae*. However, much confusion has been caused through authors like Braun (1896), Lühe (1899), Shipley (1904), Klaptocz (1906), Southwell (1913) and Cholodkovsky (1914) having based their statements on material from either the African or the Indian monitors. Beddard (1917) was the first to compare the African and Indian parasites and concluded that there are two distinct species.

A priori it would seem natural that lizards inhabiting different continents should harbour different parasites. This is the case for the species of *Bothridium*, closely related to *Duthiersia*, which is found in African and Indian pythons (see Joyeux and Baer, 1927).

Our investigations have led us to an opinion already held by most authors, namely, that there is but one species of *Duthiersia* which occurs both in Indian and African monitors. The chief differences separating the two species are of morphological interest only, and centre in the scolex. The scolex of *Duthiersia*, as in that of most Bothriocephalids, is extremely variable both in size and shape, and hardly affords a reliable means for differentiating the two species. We find it impossible to distinguish the scolices from one another. All present a more or less visible posterior pore. It has been necessary to cut serial sections to reach this conclusion, for when, as sometimes happens, the scolex is contracted, the pore is pressed against the strobila where it could never be found on whole mounts. The presence of a posterior pore has been denied by most authors; however, serial sections of not more than 8 μ in thickness can alone decide this point, as it is extremely rare to find a distinct pore on whole mounts.

The extreme variation of the scolex both in shape and size together with the presence of a posterior pore lead us to the conclusion that there is only a single species of *Duthiersia*, namely:

Duthiersia fimbriata (Diesing, 1854).

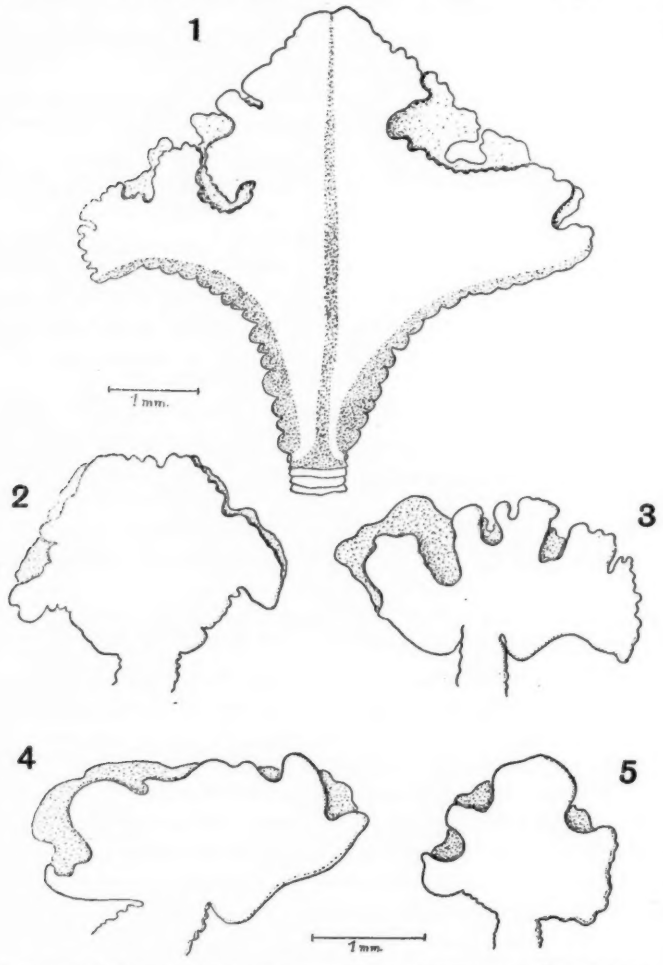
Synonyms: *Solenophorus fimbriatus* Diesing, 1854.
Duthiersia expansa Perrier, 1873.
Duthiersia elegans Perrier, 1873.
Duthiersia fimbriata (Diesing), Monticelli and Crety, 1891.

Hosts: *Varanus salvator* Laur., *V. bengalensis* Daud, *V. exanthemicus* Bosc., *V. niloticus* L., *V. albigularis* Daud.

Distribution: Southern Asia and Africa.

This species attains a maximum length of 280 mm., the average length being about 120–200 mm., although smaller specimens may be found; for instance, Klaptocz (1906) described adult specimens of not more than 82 mm. in length. The width varies from 2–5 mm. The scolex is extremely variable in shape and size as a glance at Figs. 1 to 5 will show more clearly than a lengthy description. It may be compared to two funnels joined by an intermediate band of tissue containing the main excretory vessels and the nerve stems. The edges of the funnels are frilled, giving to the whole the appearance of a flower. Both funnels open posteriorly by a minute pore, the one being situated on the ventral surface, and the other on the dorsal surface of the strobila. The pore is sometimes closely adpressed to the strobila. We have found no trace whatever of an "apical sensory organ" as described by Beddard (1917, p. 74). The width of the scolex varies from 3 to 8 mm. The segments are usually longer than wide although we have often found adult segments wider than long. The

muscular system is well developed, especially so the longitudinal muscle layer, which consists of a very great number of fibres forming a distinct layer 37 μ thick, thus separating the cortex from the medulla. The transverse muscles are represented by very fine fibres passing in between the longitudinal fibres.



Figs. 1–5. *Duthiersia fimbriata.* Different scolices showing the variation in shape.

The dorso-ventral muscles are hardly visible. The excretory system is exceedingly well developed, especially in the scolex, where it forms a complicated network. The latter is in relation with the four longitudinal vessels which pass throughout the entire strobila. The two dorsal vessels, having thicker walls, are more easily visible; they are usually 40 μ in diameter. The ventral vessels

are larger and attain a maximum diameter of 130 μ. They are united by an intricate network of secondary vessels as often found in Bothriocephalids.

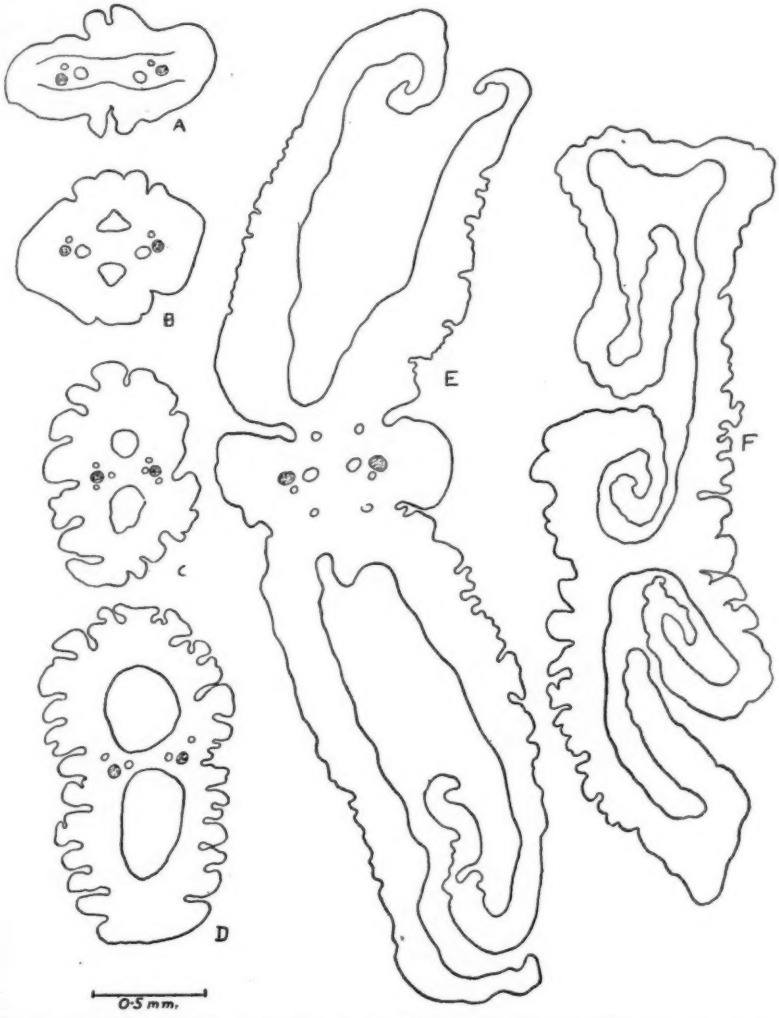
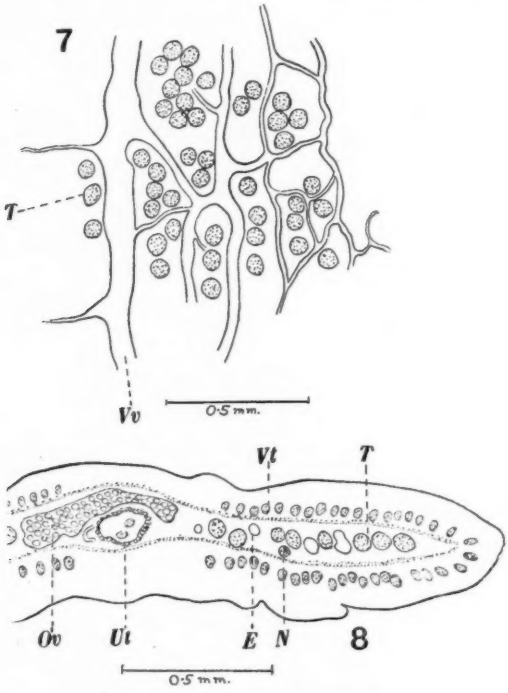


Fig. 6. *Duthiersia fimbriata*. *A–F* transverse sections passing through different regions of the scolex, *A*, being nearest the base.

The lateral nerve stems lie between the ventral and the dorsal vessels; they are 52 μ in diameter.

The male and female genitalia (Figs. 7 to 9) are of the usual Bothriocephalid type. There are about 120–160 testes occupying two lateral fields extending

beyond the excretory vessels. There are no testes dorsal to the female genitalia, but a single row of testes is to be found immediately anterior to the cirrus pouch. Each testis is about 70–80 μ in diameter. The cirrus pouch opens into a common genital atrium situated in the middle of the anterior half of the segment; it is 0·2–0·3 mm. long and 0·19 mm. in diameter. It contains besides a short cirrus a large vesicula seminis. The external vesicula seminis is very muscular and is of the usual type found in this sub-family; it measures 0·11 mm. by 0·07 mm. The vas deferens is much coiled and passes dorsally to the uterus. The vagina follows a fairly straight course in front of the uterus, passing to the



Figs. 7 and 8. *Duthiersia fimbriata.*

Fig. 7. Portion of a horizontal section showing the excretory system. *Vv.* Ventral vessel, *T.* Testes.
Fig. 8. Portion of a transverse section. *E.* Excretory vessels, *N.* Nerve stem, *Ov.* Ovary, *T.* Testes, *Ut.* Uterus, *Vt.* Vitellaria.

left or to the right of the uterine pore[1]. On reaching the ovary it forms a small receptaculum seminis which appears as a lateral swelling of the vagina. The ovary is fairly compact and is situated in the posterior half of the segment. We have never found the disposition figured by Klaptocz (1906) of the ovary in the shape of an H. The vitellaria occupy two lateral fields in the cortex. These two fields are always separated from one another by the genital area.

[1] There is a distinct sphincter vaginae.

The oviduct is surrounded by a very small shell gland and leads almost immediately into the uterus. The latter is a coiled tube opening behind the genital atrium and generally in a line with the latter. Almost the entire uterus is surrounded by uterine glands. The regions in the vicinity of the uterine pore

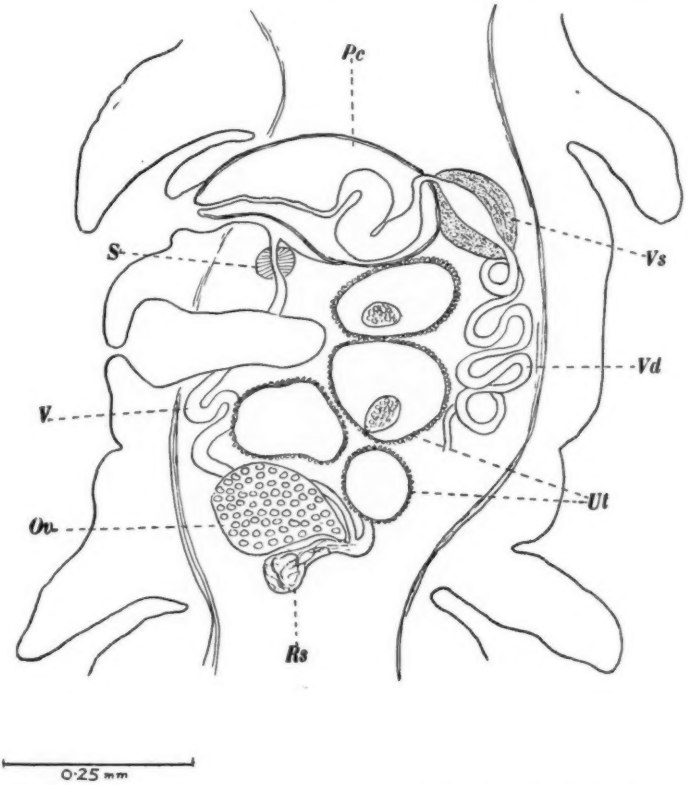


Fig. 9. *Duthiersia fimbriata.* Sagittal section. *Ov.* Ovary, *Pc.* Cirrus pouch, *Rs.* Receptaculum seminis, *S.* Sphincter, *Ut.* Uterus, *V.* Vagina, *Vd.* Vas deferens, *Vs.* Vesicula seminis.

and of the oviduct are alone devoid of glands. There are usually three to four uterine coils, the latter becoming dilated with eggs, especially in the region of the uterine pore. The eggs are operculated and measure 59–63 μ by 37–44 μ.

(*b*) **Proteocephalus niloticus** (Beddard, 1913).

Among several specimens of *D. fimbriata* from *Varanus niloticus* captured at Dakar we have found numerous fragments of *Proteocephalus niloticus* (Beddard, 1913)[1]. (Figs. 10–12.)

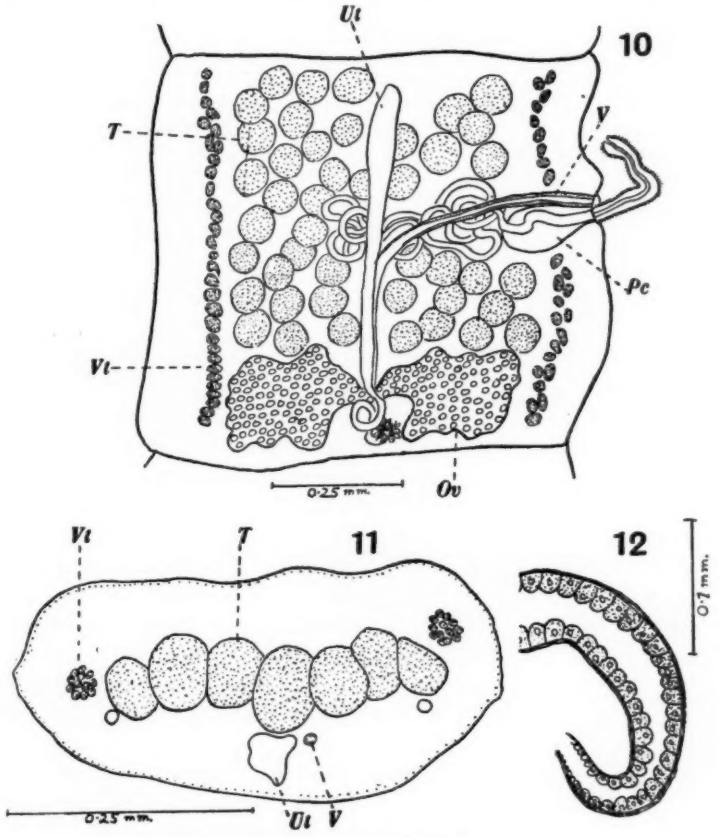
[1] We accept Woodland's revision of the genus *Proteocephalus* and consider the genus *Acanthotaenia* of biological but not of systematic interest.

This species was first described by Beddard (1913 *a*) from *Varanus niloticus* L. which died in the gardens of the Zoological Society of London. Beddard's description is totally insufficient and before comparing this species with the other known species we will first redescribe it.

It is difficult to determine the total length of this worm because of its great fragility—only fragments (the longest ca. 40 mm.) being present, but we estimate the greatest length to be at least 50–60 mm. The width of the strobila varies from 0·5–1 mm. and most of the segments are either square or longer than wide. The limit between two successive segments is hardly visible either in whole mounts or sections. The only scolex found is 0·5 mm. in diameter. The four suckers are cup-shaped and 0·13 mm. in diameter. The fifth sucker is very well developed and 0·2 mm. in diameter. The entire scolex is covered with minute spines which are also found on the suckers.

The musculature of the strobila presents several interesting features already described by Woodland (1925) for other species. The sub-cuticular musculature is well developed and consists of a single layer of stout longitudinal fibres and a layer of transverse fibres. This seems to be the only muscular system throughout the strobila. A parenchymatous musculature appears to be absent or invisible in our sections, so that the usual distinction of a cortex and a medulla cannot be made. The sub-cuticular cells are very distinct. The excretory system consists of the usual four longitudinal vessels, two dorsal and two ventral; the latter alone are visible in adult segments where most of the available space is occupied by the testes. The genital apertures alternate irregularly, the pore being situated a little in front of the middle of the edge of the segment. The cirrus pouch is 0·19–0·2 mm. in length, and 0·08–0·1 mm. in diameter. It is pear-shaped and contains several coils of the vas deferens together with a long cirrus. The latter when fully extended measures 0·37 mm. in length and 0·02 mm. in diameter and is closely set with minute cuticular spinelets. As the cirrus alone is longer than the cirrus pouch it is obvious that it will be found coiled within the latter when not protruded. The vas deferens is extremely coiled, the coils lying in a mass in the middle of the segment between the vagina and the testes. The latter occupy a single dorsal layer limited laterally by the vitellaria and posteriorly by the ovary. There are about 45–60 testes in each segment; they are large, measuring 74 μ by 60 μ. The vagina opens generally in front of the cirrus pouch although we have often found the reverse disposition. The course of the vagina is that usually found in this genus and needs no comment (see Fig. 10). Certain structural details of the vagina deserve attention. The first part, extending from the pore to the middle of the segment, is thick-walled and lined with closely set setae. The vagina passes ventrally to the testes and dorsally to the uterus and to the ovarian isthmus where it joins the oviduct. In this part it appears somewhat dilated and sections show a distinct epithelial lining whose cells measure about 8 μ and appear to be of a glandular nature. The ovary is fairly compact and measures 0·6 mm. across. The shell gland is situated behind and somewhat

dorsally to the ovary. The uterus appears as a median tube which soon shows lateral outpocketings which increase in size and finally occupy almost the entire segment. In gravid segments there are about 12–20 lateral branches to the uterus. The vitellaria are situated on both sides of the segment and reach



Figs. 10–12. *Proteocephalus niloticus.*

Fig. 10. General anatomy.
Fig. 11. Transverse section.
Fig. 12. Terminal portion of the vagina showing the glandular cells.
(Lettering as under Figs. 1–9.)

as far back as the ovary. The follicles are about 11 μ in diameter. The ripe ova, *i.e.* those with a distinct shell, are spherical and measure 23–27 μ in diameter. The embryo measures 15 μ in diameter.

Up to now ten species of Proteocephalids have been described from monitors; they are *P. articulatus* (Rudin, 1917), *P. continuus* (Rudin, 1917), and *P. niloticus* (Beddard, 1913) from African monitors, *P. beddardi* (Woodland,

1925), *P. shipleyi* (Linstow, 1903) and *P. woodlandi* (Moghe, 1926) from Indian monitors and *P. biroi* (v. Ratz, 1900), *P. saccifer* (v. Ratz, 1900), *P. tidswelli* (Johnston, 1909) and *P. varius* (Beddard, 1913) from Australian monitors.

If we now compare the three above-named species from the African monitor *Varanus niloticus* L. we see at once that they are closely related, the following table facilitating comparison:

Species	Length mm.	Width mm.	Number of testes and size μ	Size of cirrus pouch in mm.
P. articulatus	75	0·5	50–70, 65	0·18 by 0·07, cirrus armed
P. continuus	200	1	60–80, 80	0·14 by 0·08, cirrus armed
P. niloticus	50–60	0·9	45–60, 67	0·19 by 0·08, cirrus armed

There seems to be no doubt as to the identity of *P. niloticus* and *P. articulatus*. The slight difference in the measurements are due to specific variability. The distribution of the testes is identical in both species and the average diameter of a single testis is nearly the same. We therefore consider *P. articulatus* (Rudin, 1917) as a synonym of *P. niloticus* (Beddard, 1913). On comparing *P. niloticus* with *P. continuus*, the latter is found to differ from the former only in a few details, *i.e.* mainly in the size of the strobila, the testes being larger and more numerous, and the cirrus pouch being smaller. Are these differences sufficient to distinguish the species? Provisionally, *P. continuus* may be considered a good species until more material has been examined although it may well be but a *forma major* of *P. niloticus*. With regard to the Indian species, *i.e. P. beddardi* (Woodland, 1925), *P. shipleyi* (Linstow, 1903) and *P. woodlandi* (Moghe, 1926), *P. shipleyi* was described by Linstow (1903) from a single specimen with undeveloped uterus. His description is totally insufficient and renders any comparison difficult, yet we feel convinced that further study will show that *P. woodlandi* Moghe, 1926 is synonymous with *P. shipleyi*; *P. beddardi* on the other hand, although closely related to the preceding species, seems to present sufficient specific details to justify its systematic position.

Therefore the Indian and African monitors harbour very closely related species of the same genus of tapeworms. Further study on more ample material will no doubt prove that the Indian and African monitors harbour the same species of parasites.

The four species described from Australian monitors, viz. *P. biroi* (v. Ratz, 1900), *P. saccifer* (v. Ratz, 1900), *P. tidswelli* (Johnston, 1909) and *P. varius* (Beddard, 1913)[1], are also closely related, although it is impossible to compare them fully because *P. biroi*, *P. saccifer* and *P. tidswelli* are insufficiently known. We are however convinced, that when more material has been examined, similar results to those above described will be obtained, and that the number of purported species of Proteocephalids from Australian monitors will be much reduced.

[1] We do not agree with Woodland (1925, p. 379) when he states that the worm described by Nybelin (1917) is specifically different from that described by Beddard (1913).

Woodland (1925) has recently given a list of the known species of *Proteocephalus* inhabiting the *Varanidae*; to this list we add *P. continuus* (Rudin, 1917).

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NOTE ON THE ACTUAL APPEARANCE OF A CESTODE SHELL-GLAND.

By WILLIAM P. CANAVAN.

University of Pennsylvania.

(With 1 Text-figure.)

Authors almost invariably figure the shell-gland of Cestodes in a form resembling that of a daisy's corolla. This may serve to indicate the position of the shell-gland but it should be remembered that the shell-glands are not all of this form. Thus, the shell-gland shown in Fig. 1 is somewhat shrub-like in

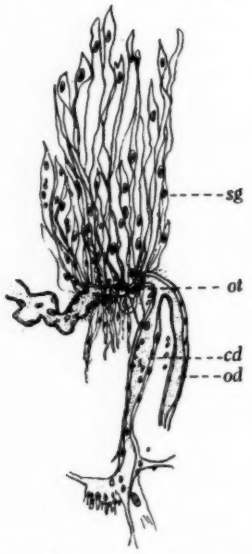

Fig. 1. Shell-gland from *Phyllobothrium ketae*, a marine fish cestode. Host: *Oncorhynchus keta* Walbaum, the dog salmon. (Scale = ·05 mm.)

form, consisting of irregularly fusiform elements which grow filamentous at one end and they are clustered on one side of the oötype of the uterine duct. The nucleus is situated in the fusiform portion and the filamentous portion extends toward the parenchyma of the cortical layer.

Attention is drawn to the inadvisability of depicting the shell-gland in a schematic manner, like a daisy blossom, because this may lead to confusion in the determination of species.

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NOTE ON A HAEMOGREGARINE IN *LEPIDOSIREN PARADOXA*.

By MARGARET W. JEPPS.

Lecturer in Zoology, Glasgow University.

(With 7 Figures.)

The purpose of this note is to record the occurrence of a haemogregarine parasite in the red blood corpuscles of the South American lung-fish, *Lepidosiren paradoxa*.

One of the very large erythrocytes of this fish is depicted in Fig. 1. It exhibits the structure described by Bryce (1904), and in addition a number

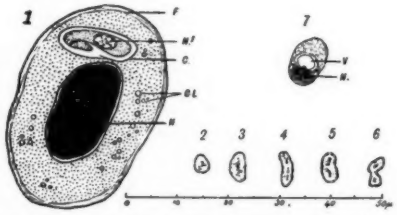

Fig. 1. Infected erythrocyte from L. (ventral) pulmonary artery. Smear, stained Delafield's haematoxylin. *F*, fibrillar marginal zone; *Gl*, globules in cytoplasm; *N*, nucleus of erythrocyte; *N'*, nucleus of haemogregarine; *C*, capsule of haemogregarine.

Figs. 2–6. Nuclei from haemogregarines. Figs. 2, 3, 5 and 6 from similar preparations to the last. Fig. 4, from a section of the L. lung, stained as before. Fig. 6 is from a short broad haemogregarine, ? stage in division.

Fig. 7. Oval parasite from erythrocyte in posterior vena cava (in liver). Smear, stained Heidenhain's iron haematoxylin. *V*, vacuole with solid contents; *N*, nucleus.

of globules of unknown nature in the cytoplasm. These globules seem to be constantly present in the fresh blood corpuscles[1], and in smears of formalin-fixed material, from adult fish. In sections they are not so readily seen. The haemogregarine[2] lies coiled in its capsule at the side of the erythrocyte nucleus, which is not displaced. As many as four parasites are commonly seen in a single infected corpuscle. Some are of the long slender bent form shown in the figure, while others are shorter, broader, and not bent. The somewhat variable structure of the nucleus is shown in Figs. 2 to 6. There is no definite

[1] I have had the opportunity of examining the blood of five Lepidosirens living in the Zoological Laboratory of Glasgow University through the kindness of Prof. J. Graham Kerr. None of these were found to be infected with haemogregarines.

[2] Probably somewhat shrunken.

karyosome. Fig. 7 represents an oval parasite, apparently with no capsule, from an erythrocyte which also contained a haemogregarine of the usual form. A similar oval was seen free amongst a mass of debris in another smear (cf. Minchin and Woodcock, 1910 and Henry, 1913 *a*, Pl. VIII, figs. 1–10).

No other recognisable stages were seen in smears of blood from the left (ventral) pulmonary artery, and the posterior vena cava; or in sections of lung and liver from the single infected specimen I have studied. This was the anterior end of one of Prof. Kerr's specimens preserved in formalin several years ago. My attention was first drawn to the parasite by Dr Tudor Jones, who brought me an infected red blood cell for diagnosis.

Prof. J. S. Dunkerly very kindly tells me that he observed haemogregarines in the red blood corpuscles of 10 out of 14 Lepidosirens collected by Prof. Agar (*i.e.* 71 per cent.), when he was examining them for the Myxosporidian *Agarella gracilis* (Dunkerly, 1925).

Although haemogregarines have long been known to occur in the blood of fishes[1] we are still almost entirely ignorant of their life cycle in these hosts. Only some stages of schizogony have so far been described (Laveran and Mesnil, 1901, and others). Reichenow (1910) and Robertson (1910) have worked out the life-history of haemogregarines in tortoises and in the leeches which feed upon them, and it is with their accounts that the fragmentary observations on the haemogregarine of *Lepidosiren* must be compared. As far as these go there is agreement in details of structure etc. between the parasite now described and their accounts of species of *Haemogregarina*. I can find no mention of a haemogregarine in *Lepidosiren* in the literature[2], and therefore propose to name it provisionally *Haemogregarina lepidosirenis*, in accordance with the usual practice in the case of haemogregarines showing no distinctive feature save their habitat in a particular host.

It would seem that the infection is not uncommon, but since *Lepidosiren* is one of the few survivors in a rapidly changing country of a formerly large group of archaic fishes, and may itself become extinct comparatively soon, it seems desirable to place the above observations on record.

I have much pleasure in thanking Prof. Graham Kerr for allowing me to make use of his valuable specimens of *Lepidosiren*, both alive and preserved; I am once again indebted to him for reading and criticising what I have written, and to the late Mr P. Jamieson for cutting my sections.

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[2] *v.* Migone (1916).

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FURTHER OBSERVATIONS AND EXPERIMENTS ON THE CULTIVATION OF *ENTAMOEBA HISTOLYTICA* FROM CYSTS.

By CLIFFORD DOBELL, F.R.S.

(*National Institute for Medical Research, London, N.W. 3.*)

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I. Introductory.

In a recent paper[1], written jointly with Dr P. P. Laidlaw, methods were described for cultivating *Entamoeba histolytica*, and various other entozoic amoebae of man and monkeys, from the encysted forms. We there showed that cultures of all the species studied can be readily obtained by merely incubating such cysts in any of the media suitable for the cultivation of the corresponding active forms—provided that certain precautions are taken.

Whilst our paper was in the press, one by Yorke and Adams (1926), in which somewhat similar findings were reported, made its appearance. These authors apparently take it for granted that they were the first to cultivate *E. histolytica* from its cysts; but they have evidently overlooked an earlier announcement of ours. It is true that their paper (received for publication on June 24th, 1926) was issued on August 13th, 1926; whereas ours—whose publication was delayed by the General Strike in May—was not published until a month later than theirs (September 14th), though it was received by the Editor more than a month earlier (May 18th). But in the *Report of the Medical Research Council* for 1924–1925 (dated 1925, but actually published on January 22nd, 1926) it had already been recorded (pp. 31, 32) that

[1] Dobell and Laidlaw (1926).

Dr Laidlaw and I had been able to obtain *in vitro* all stages in the life-history —including encystation and excystation—of *E. histolytica* (from man), and of two entozoic amoebae of monkeys. It was also there put on record that we had repeatedly obtained cultures by hatching the cysts of all three species[1]. As Yorke and Adams state (at the end of June, 1926) that they had been studying this subject for the previous six months, it is therefore clear that they were only beginning their work at the time when the preliminary announcement of our success—based on a year's work—was published.

For the sake of historic accuracy I must add that the encystation of *E. histolytica* was first obtained *in vitro* by Dr Laidlaw on December 1st, 1924; and that I first hatched the cysts of this species, and so obtained cultures from them, early in the same month—as stated in our previous paper. This is well known to many of our colleagues, and can be attested by Dr Boeck, Dr Drbohlav, and many other workers who were informed of our results at the time or soon after. It is probable, however, that cultures were first made from the encysted forms of *E. histolytica* by Boeck and Drbohlav at the beginning of March, 1924[2]. We do not therefore claim to have been the first in the field, though there can be no doubt that we had discovered how to cultivate *E. histolytica* and several other entozoic amoebae from cysts long before Yorke and Adams made any similar attempts.

Since I first obtained cultures from the cysts of *E. histolytica* in December, 1924, I have been almost continuously engaged in working out the details, and in trying to eliminate the numerous sources of error involved in making and interpreting such experiments. But the work is laborious and beset with all manner of pitfalls; and consequently I am still, after more than two years' study, in doubt about certain developmental details. I hope, however, to be able to publish shortly a full and correct description of the whole life-history of *E. histolytica*—and of several other entozoic species—as observable *in vitro*.

The present paper is, as will be evident, merely an elaboration of the work previously reported in conjunction with Dr P. P. Laidlaw. Although I alone am responsible for the experiments here described, and for their presentation, I have had the benefit of his advice and criticism throughout. He has seen most of the experiments in progress, and witnessed their results; and I have his permission to say that he agrees with my present conclusions when they happen to contradict our previously reported findings. I gratefully acknowledge my indebtedness to Dr Laidlaw for his continued collaboration.

Before recording or discussing any of my experimental results, I would make a few general observations. They are of importance for the understanding of what follows.

[1] Wenyon (1926), whose book appeared before the paper by Yorke and Adams was published, duly notes (Vol. I, p. 227) our success in hatching and cultivating "*E. nuttalli*," but curiously enough makes no mention of the fact that we had been equally successful with *E. histolytica* from man—recorded in the very paragraph to which he alludes.

[2] See Boeck and Drbohlav (1925), p. 385.

II. General Considerations.

(1) Whenever it is desired to obtain cultures from the *cysts* of amoebae, it is obviously necessary to make absolutely certain that the original material containing the cysts does not contain living amoebae also. The accidental presence of free forms, along with the cysts, is a fertile source of error in any such experiments.

For example: if it be desired to ascertain whether the cysts of *E. histolytica* are capable of hatching (and so producing cultures of amoebae) immediately after leaving the body, it is not possible to answer this question by merely washing and concentrating the cysts from a fresh stool in which no amoebae can be seen, and then at once inoculating this material into culture-medium and incubating it. If amoebae appear in the culture later, they may be—and often are—derived not from the cysts but from amoebae accidentally introduced with the inoculum.

It is obviously no answer to this objection to say that amoebae were not discoverable microscopically in the original stool or in the cyst-concentrate: because it is certain that amoebae *are* sometimes present among such cysts (as more exhaustive microscopic examination sometimes—though not always—reveals); and it not seldom happens that *E. histolytica* (and other amoebae) *can* be cultivated from faeces and other material in which neither cysts nor amoebae can be found with the aid of the microscope alone. A culture frequently supplies a more accurate test of the presence of amoebae than a microscopical examination.

(2) In the experiments described by Yorke and Adams (1926, 1926 *a*) this source of error appears to have been wholly disregarded, and some of their findings are therefore open to serious criticism. From their descriptions it is not certain that all the amoebae which they obtained in their cultures were derived from cysts of *E. histolytica*: it is not even certain that all the amoebae which they found and figured really belong to this species—since they adduce no evidence to prove that the patients from whom their cysts were obtained were infected with *E. histolytica* alone. Many of the amoebae which they describe—such as the multinucleate giant forms (which are undoubtedly abnormalities)—may appear in cultures both of *E. histolytica* and of *E. coli*. When a culture is made from cysts in faeces by their methods, one may be sure that those amoebae which are actually seen to emerge from cysts are derived from this source: but as regards other amoebae found in the same culture there can be no such certainty.

(3) In order to eliminate the source of error just noted, Laidlaw and I adopted two different methods which we have already described[1]. After first making sure that we were dealing with a single known species, we either kept the mixed amoebae and cysts for a (supposedly) sufficient time at room-temperature to kill off all free forms; or we treated the mixture with dilute

[1] Dobell and Laidlaw (1926).

hydrochloric acid so as to kill all amoebae without injuring the cysts. By such means we were able to make certain that the cultures which we obtained from cysts were really derived from cysts alone, and not—either wholly or in part—from free amoebae (possibly some of them of other species) accompanying them accidentally.

(4) As a result of many experiments, we concluded that the free amoebae of *E. histolytica* (and all other species which we studied) were usually incapable of surviving at room-temperatures for more than about a day; and we therefore always kept a mixture of amoebae and cysts for at least two days before we attempted to make cultures from the cysts. We also found that the free amoebae were invariably killed by exposure to $N/20$ HCl for more than half an hour at room-temperature (usually much sooner), while the cysts could withstand this concentration for several hours: and we therefore treated our cysts for a minimal time of one hour with acid of at least this concentration before inoculating them into culture-tubes.

(5) It will be clear, from the foregoing, that it is of prime importance to ascertain (*a*) the exact time for which free amoebae can survive at room-temperatures; and (*b*) how long they can withstand, at the same temperatures, a concentration of HCl equal to $N/20$. I have made very many experiments to determine both these points, and will therefore summarize them in the following pages. I have found that our original statements require correction in one important particular.

(6) It is furthermore evident that, when cultures are made from cysts which have been kept at room-temperature in order to kill off any concomitant amoebae, the cysts themselves are necessarily cooled also, and allowed to remain at this lowered temperature for a certain period of time. Is this cooling and keeping necessary, in order that they may hatch? Dr Laidlaw and I stated that it was. We said that the necessary conditions for hatching the cysts of *E. histolytica* consisted in cooling them and keeping them cooled for about a day before inoculating them into suitable medium. Our statement was based upon experiments which I have repeated and varied and finally found fallacious. I shall therefore describe the more important of them in some detail.

(7) It is necessary, for various purposes, to ascertain how long the cysts of *E. histolytica* can survive outside the body. Yorke and Adams (1926 *a*) have attempted to determine this point, and I have devoted much attention to it during the last two years. My findings do not agree with theirs, and I shall therefore record them.

(8) Cultural experiments made to determine the resistance, or time of survival, of amoebae or cysts when exposed to various influences, necessarily involve the making of various "positive" and "negative" cultures—from which the final conclusions are drawn. For example: if the time of survival of *E. histolytica* cysts outside the body is to be determined, we can take a batch of such cysts, and sow and incubate a sample in culture-medium at

daily intervals, and thus ascertain the latest date at which a positive culture is obtainable; and from this we can infer the number of days for which the cysts remain alive. In such an experiment a great deal obviously turns upon the value to be attached to a "negative" culture: but "negative" cultures are only too easily obtainable by using unsuitable culture-medium or inappropriate technique, and it is thus necessary to make sure that the most favourable methods of cultivation are employed throughout, and to check all negative findings whenever possible. This is, no doubt, self-evident: but as I have often been deceived by "negative" experiments—which, by improved technique, have later been found really positive—I shall record some of my experiences for the information of others.

The questions raised in the foregoing paragraphs are all difficult to answer correctly, though they *can* all be answered by appropriate experiments. I have found answers to all of them, but do not claim that they are final. I hope that other workers, by repeating my experiments and varying them in ways which I have not thought of, will soon be able to verify or contradict my conclusions, so that we may arrive at the truth as quickly as possible. I am fully aware that finality in such matters can only be reached by the work of many.

In the ensuing section I shall put the chief questions at issue in a concrete form, and give the experiments which I have made in order to answer them. I shall then draw my own conclusions from the experimental findings, and leave it to others to judge how far the experiments are sound and my interpretations of them justifiable.

III. Experimental.

Before describing any experiments I would note a few points which are—to my mind—important, and which may explain certain discrepancies between my findings and those of others.

I here use the term *Entamoeba histolytica* not only to denote the well-known human parasite of this name, but also for the very closely related organisms which occur in monkeys[1] (*Macacus rhesus* and *M. sinicus*). At present I am unable to distinguish these forms by any valid systematic character, and regard them all as strains or varieties of a single species. I therefore beg the question of their specific identity. (I hope to be able to publish much evidence on this head shortly.) Most of my work has been done with simian strains—to which my conclusions, therefore, specially apply.

In making cultural experiments, I have always taken care to use "pure" cultures, and culture-medium which I have found—by previous trials—to be specially suitable for the growth of the particular strain studied[2]. Negative cultures in unsuitable medium are obviously insignificant.

[1] Cf. Dobell (1919); Dobell and Laidlaw (1926).

[2] Cf. Dobell and Laidlaw (1926). By "pure" cultures, I mean pure as regards the amoebae. The accompanying bacteria have not been comprehensively determined.

I have found, by repeated experiment, that "negative" primary cultures made from intestinal amoebae or their cysts—even when the most favourable medium is used—are not always conclusive. (This applies especially to cultures made from faeces—always of unknown bacterial content.) Whenever a primary culture is negative, therefore, I now make a subculture from it—at an appropriate time, which has to be determined for each strain studied—into suitable medium; and I call no result "negative" unless both primary culture and subculture show no amoebae. This precaution has converted "negative" into positive results in a significant proportion of experiments; and neglect of it has vitiated many of my earlier findings, which I therefore now regard as inconclusive and not worth recording. In the following pages, accordingly, a negative result is given, as a general rule, only when it has been established by means of a culture and a subculture[1] made in medium previously proved to be highly favourable for the growth of the particular strain employed.

Whenever, in the following pages, I refer to "room-temperature," I mean the fluctuating temperature of my own laboratory. My experiments have been carried out at all seasons during the past two and a half years, and the temperature has ranged from 10° C. to 20° C. Occasionally it has exceeded these limits. But as experiments made during hot and cold periods have given essentially identical results, I have not attempted to define the temperature-conditions more exactly.

Moreover, when I speak of cultures as "normal" and "healthy," I mean that the amoebae in them displayed their typical structure—both alive and in good cytological preparations—and were capable of propagation for any desired period by suitable methods of cultivation. In all doubtful cases stained preparations have been made and examined, and the cultures continued for a time sufficient to make certain that they could be maintained *ad libitum*.

Most of the experiments about to be described have been made with organisms in cultures, for it is impossible—at present—to make similar experiments with cysts or amoebae directly derived from stools. It was therefore necessary, in the first place, to discover some method of obtaining amoebae or cysts—as required—*in vitro*. This I have at last succeeded in doing, in the case of at least one strain of *E. histolytica*[2]: so that I can now at any time obtain amoebae in any quantity; make them encyst or excyst at will; and thus have unlimited material of all stages in the life-cycle at my disposal for experimental purposes. Without such material I could not have performed many of the experiments here described.

[1] If the subculture from a negative culture is likewise negative, I do not usually test it further: for I have never yet obtained a positive result—after numerous trials—by attempted further subcultivation of negative subcultures.

[2] Strain *K.* 28 *C.*, derived originally from *Macacus sinicus*. It was passed through a kitten (in which it produced acute amoebic dysentery), and then recovered in culture from this animal. It will be described in detail elsewhere.

(1) *How long can the amoebae of* E. histolytica *survive at room-temperatures?*

At first sight this may appear an easy question to answer; but I have found it extremely difficult. It is only necessary, apparently, to take some amoebae; keep them at room-temperature for different times; and then examine them microscopically and inoculate them into suitable media, in order to find out how long they remain alive. The difficulty lies in obtaining amoebae *only*—unmixed with cysts—for experiment.

It is, in my experience, impossible to make any satisfactory experiments of this sort with amoebae passed in the stools. No two stools are exactly alike (in bacterial content, or in other ways), and it is impossible absolutely to exclude the presence of cysts in human or simian faeces by mere microscopic examination. A single viable cyst, if present undetected among the amoebae used for an experiment, is sufficient to vitiate all results.

It occurred to me that amoebae in the dysenteric discharges of experimentally infected kittens might supply more suitable material for investigation—since cysts are never formed by *E. histolytica* in this host. But I have abandoned this line of work as hopeless: for I have obtained positive cultures from such material (in which no amoebae were discoverable microscopically) after keeping it for several hours, and negative cultures from dysenteric stools—containing microscopically visible amoebae—inoculated into culture-tubes within a few minutes of being passed. The results of such experiments are too uncertain and irregular to be of any real value, because intestinal contents—of unknown bacterial and chemical composition—on leaving the body undergo changes which cannot at present be accurately defined, controlled, or predicted.

But since it is now possible to obtain amoebae in unlimited numbers in culture, it seems feasible to experiment with such organisms. In certain media—for example, that devised by Boeck and Drbohlav—*E. histolytica* does not usually encyst[1]: at least, no cysts can usually be discovered by exhaustive microscopic examination. I therefore attempted to ascertain how long *E. histolytica* (both human and simian strains) could survive in the amoeboid form, at room-temperature, by experimenting with amoebae grown in Boeck-Drbohlav medium. I took young and healthy cultures, and set them on my bench; and at various times afterwards I then made subcultures from them into favourable media. The results, with all strains, were fairly consistent; but now and then I obtained positive cultures which were unexpected, and difficult to reconcile with the rest.

The following experiments, made with cultures of a strain (*K.* 28 *C.*) of *E. histolytica* from *Macacus sinicus*, will serve as an illustration. This strain grows extremely well in egg-serum medium[2], but requires transplantation

[1] Cf. Boeck and Drbohlav (1925); Dobell and Laidlaw (1926).

[2] This is the medium called "Ehs" in our previous paper (Dobell and Laidlaw, 1926).

every second or third day. A 24-hour culture always contains abundant amoebae: and no cysts had ever been found microscopically in such cultures up to the time when these experiments were made. Thirteen 24-hour cultures were prepared, on different occasions, and then placed at room-temperature. After various intervals of time the sediment in each tube was inoculated into fresh medium, and incubated and tested—all negative cultures being subcultivated to confirm the diagnosis[1]. The final result of this series of experiments[2] is shown in Table I—each sign (+ or −) here denoting a separate experiment.

Table I. *Amoebae (in cultures) kept at room-temperature.*

Time (in days)	1	2	3	4	8
Subcultures	+	+	−	−	−
	+	+	−	.	.
	.	−	−	.	.
	.	+	+	.	.
	.	−	.	.	.

These 13 experiments appear to show that individual amoebae may occasionally survive for as long as 3 days, but not longer: and careful microscopic examination clearly indicated—as usual—that the majority were certainly dead within 48 hours.

I drew this conclusion at first, but came to doubt its validity when I subsequently obtained a positive result from a similar 24-hour culture of amoebae which had been standing on my bench for 7 days. As it seemed to me incredible that any amoeba of this species could survive for this time under such conditions, I concluded that this result must have been due to the presence of undetected cysts in the original culture. But if cysts were present in it, they might also have been present in any of the others: and if so, this would obviously vitiate all the findings.

From certain other experiments I had reason to believe that cysts (though I could not find any by microscopic examination), if accidentally present among the amoebae, would probably be most numerous on the second or third day of incubation. Accordingly, I made four similar cultures, in the same medium, but incubated three of them for 2 days and one of them for 3 days before placing them at room-temperature. One 2-day culture was tested after being at this temperature for 3 days: the others were kept for a full week before being subcultivated. The result, in every case, was positive. I was therefore convinced that my previous findings were untrustworthy, since undetected cysts were possibly present among the amoebae tested: and I was subsequently able to confirm this supposition by actually finding a few

[1] The medium used for these tests was inspissated horse-serum with Ringer-eggwhite and starch (Dobell and Laidlaw, 1926), in which this strain grows readily and luxuriantly.

[2] In this and later experiments, no results are given for amoebae kept at room-temperature for less than 24 hours. This is because earlier work, with many different strains, had shown that the amoebae in healthy cultures frequently survive exposure to this temperature for any time up to about a day (Dobell and Laidlaw, 1926).

fully-formed cysts[1] among the amoebae in a 2-day culture of this strain grown under identical conditions.

It is thus clear that the problem cannot be solved unless cultures which can be *proved* to contain *amoebae only*—never cysts—are used for such experiments. Ordinary "cyst-free" cultures are not trustworthy.

It occurred to me that cultures containing amoebae only might be obtained by beginning them from cysts. If cultures are prepared by hatching the cysts of *E. histolytica*, abundant amoebae can be obtained—by suitable technique[2]—and these amoebae multiply rapidly for a time before they encyst again. I have never found cysts in any cultures so made before the lapse of two days from the time of hatching: and I therefore repeated my previous experiments with cultures prepared from cysts, and incubated for only 24 hours.

The cysts used for all these experiments were themselves obtained in cultures[3]—by the employment of a special technique—and were then kept for about a week (6–8 days) at room-temperature before inoculating them into fresh medium. (This was done to insure that any concomitant amoebae would die.) Very rich cultures were obtained after 24 hours' incubation, and the amoebae were then put at room-temperature and later inoculated into fresh medium at varying intervals of time. On incubating these subcultures, the results shown in Table II were obtained (in a series of 13 experiments)—each sign (+ or −), as before, indicating the result of a separate experiment.

Table II. *Twenty-four-hour cultures of amoebae (from cysts) kept at room-temperature.*

Time (in hours)	24	30	35	48	54	72	96
Subcultures	+	+	+	−	+	+	+
	+	.	.	−	.	.	.
	+	.	.	−	.	.	.
	.	.	.	+	.	.	.
	.	.	.	+	.	.	.

These results appear to show that *some* amoebae survived at room-temperature for as long as 4 days—a result which is hardly credible. For a time I was unable to understand the last three positive findings: but it then occurred to me that they might possibly be due to the presence of unhatched cysts carried over from the original culture. It seemed possible that some of the original cysts had not hatched after 24 hours' incubation, but had remained alive and—after cooling again—had hatched on further incubation. This seemed to me unlikely[4], but I determined to test the possibility by further experiments.

[1] These cysts were kept, and successfully hatched after remaining a week at room-temperature.

[2] See Dobell and Laidlaw (1926), p. 297.

[3] The bacterial composition of the cultures was therefore a constant factor throughout. It is impossible to make any consistent experiments of this sort with cysts derived from faeces, as the cultures so obtained are of unknown and variable bacterial content. Regarding the influence of the bacterial constituents of amoebic cultures, see Dobell and Laidlaw (1926), p. 307.

[4] The maximum time taken to hatch—in *E. histolytica*—when the cysts are incubated under highly favourable conditions, I have not yet determined with precision. But I have in one instance seen a cyst which did not hatch until it had been incubated for 17½ hours.

In order to make certain that *all* the hatchable cysts in the original cultures had really hatched, I prepared a second series of cultures as before, but incubated them not for 24 hours but for 42–48 hours. (I was afraid to incubate them longer than 2 days, lest some of the hatched amoebae might again encyst, and so vitiate the results.) Excellent cultures, in which abundant amoebae but no discoverable living cysts were present, were thus obtained. Testing these amoebae, in the same way as before, I obtained the results shown in Table III (11 experiments).

Table III. *Forty-two to forty-eight-hour cultures of amoebae (from cysts) kept at room-temperature.*

Time (in days)	1	2	3	4
Subcultures	+	+	+	−
	+	+	+	−
	.	−	+	.
	.	.	−	.

If my reasoning is sound, this shows that amoebae can live for 3 days at room-temperature, but not for 4. I believe this to be correct, as it agrees with all other experiments in which I was able to exclude the presence of cysts with a degree of probability amounting almost to certainty. For example, I made a further series of experiments along somewhat similar lines; but instead of incubating the original cultures (from cysts) for 2 days, I incubated them for 24 hours only, and then made subcultures from each. These I again incubated for 24 hours, when they contained abundant amoebae (and almost certainly no cysts) which I treated as before. Ten experiments made in this way showed conclusively that the amoebae on exposure to room-temperature all died long before the end of the third day.

In the course of these and similar experiments, it became evident that the amoebae in cultures containing starch are much more resistant to cooling than those in starch-free media. All the amoebae which survived for 3 days at room-temperature in the foregoing experiments were fed on starch; but up to the present I have never obtained a positive culture from amoebae cultivated in Boeck-Drbohlav medium (without starch) after exposure to room-temperature for more than 48 hours. It therefore seems probable that the state of nutrition of individual organisms determines, in part, their ability to withstand cooling.

All the experiments just recorded were made with cultures of *E. histolytica* from *Macacus sinicus* (Strain *K.* 28 *C.*), but they have been checked by numerous others with strains from man and from *M. rhesus*. For example, my oldest strain (*D*) of *E. histolytica* (from man), which has now been cultivated for about two and a half years, has long since ceased to form cysts in most cultures, though it still grows luxuriantly in media containing starch[1]. But I can usually recover the amoebae (by cultivation) from such cultures after cooling for 24 hours, often after cooling for 2 days, and occasionally

[1] See Dobell and Laidlaw (1926).

after cooling for 3 days[1]. When, however, the cultures are kept at a low temperature for a longer period, they almost invariably give negative results when subcultivation is attempted. (Rare positive results obtained after 3 days are probably due to the presence of undetected cysts.)

I have found that *E. histolytica* amoebae can survive at room-temperature for similar periods if they are kept in Ringer's fluid instead of being left in the culture-tube. Some individuals can also live for at least a day in distilled or tap water, though the majority usually die much sooner under such conditions.

I am now satisfied, therefore, from all my experiments[2], that the amoebae of *E. histolytica* are able to survive at room-temperatures—under otherwise favourable conditions—for any period of time up to 3 days: but I have no evidence that they can ever live longer, in such circumstances, and the vast majority of individuals certainly cannot live nearly as long.

It follows, from this, that whenever one desires to make cultures from *cysts* of *E. histolytica*, and uses for this purpose faecal or cultural material which may conceivably contain amoebae as well as cysts, it is necessary to cool it (unless other methods are used for killing the free forms) *for more than 3 days* in order to exclude all possibility of error. Free amoebae usually die long before this time; but it seems certain that isolated individuals *can* survive for any time up to 3 days at ordinary room-temperatures.

A correlative question, which also—for some practical and theoretical problems—requires an answer, is: How long can the amoebae of *E. histolytica* live at body-temperature? It has already been recorded (Dobell and Laidlaw, 1926, p. 296) that we found they could live, without multiplying or encysting, in Ringer's fluid and solid rice-starch for as long as 3 weeks. (The presence of cysts, in such "cultures," is excluded; for the amoebae gradually die in them, and I have found that cysts survive at 37° C. for not more than a few days at most.) I have one isolated experiment, however, which shows that the amoebae can live at body-temperature even longer.

The amoebae from a rich culture of *E. histolytica* (Strain *J*, from *M. rhesus*[3]) were transferred—with their accompanying bacteria—to a tube of warm Ringer's fluid and starch. This "culture" was left in the incubator (at 37° C.)

[1] In October and November, 1926, I sent—at his request—3 cultures of this strain (containing amoebae only, so far as I could ascertain) by ordinary post to Dr J. Rodhain at Brussels. They took between 2 and 3 days in transit—during cold weather—but he reports that he succeeded, nevertheless, in recovering the amoebae from one culture and in continuing the strain. Culture-cysts of Strain *K*. 28 *C*. which I sent him at the same time he was able to hatch, and obtain good cultures from, without difficulty.

[2] These now run into hundreds, only a small selection being here cited. I am fully aware that they are not as yet sufficient from a statistical standpoint. But I would remind the critical reader that the making of such experiments is extremely laborious, and takes a long time. For example, the experiments with amoebae from *M. sinicus*—cited above—though only about 60 in number, involved making and carefully examining over 200 cultures, and occupied several months—apart from all the preliminary work necessary to obtain the requisite material.

[3] See Dobell and Laidlaw (1926).

and tested at weekly intervals by microscopic examination and by inoculating a small amount of its sediment into suitable subcultures. The amoebae gradually disappeared, but positive subcultures were obtained up to the end of the fifth week (35 days).

It is clear, therefore, that some amoebae can survive at body-temperature —under certain conditions—for as long as 5 weeks.

(2) *How long can the cysts of* E. histolytica *survive at room-temperatures?*

This is a much easier question to answer than that just propounded. It requires only time and labour, and appropriate methods of cultivation.

In our previous paper (Dobell and Laidlaw, 1926, p. 304) we stated that the cysts of *E. histolytica*—both human and simian strains, from faeces or from cultures—will live at room-temperature, and remain hatchable, for a period of approximately a fortnight: and this is true whether they are kept during this time in distilled water or tap-water, Ringer's fluid or physiological saline, or in the culture-tube in which they were formed. As I have now demonstrated this by hundreds of experiments with dozens of different strains, I take it to be a fact. If the cysts are mature and healthy, I can always obtain cultures from them—barring accidents—at any desired time during the first fortnight.

But 14 days is by no means the longest time for which the cysts of *E. histolytica* can live at room-temperature. Many can survive much longer, and I have often made good cultures from cysts kept for more than 3 weeks. If a batch of cysts is tested culturally at daily intervals, the results usually become irregular during the third week, and only occasionally is a positive culture obtained during the fourth. The older the cysts, the less likelihood is there of cultivating amoebae from them; and microscopic examination shows that the great majority are certainly dead after keeping for 20 days.

Cultures are, however, a more exact test of viability than microscopic examination, as the following experiment illustrates. A batch of cysts (*E. histolytica*—a strain (*B*) pathogenic for kittens) from the faeces of *Macacus sinicus*, was carefully concentrated and washed and then kept in sterile distilled water at room-temperature. Cultures inoculated with samples of these cysts at 2-day intervals gave, from the 18th to the 28th day[1] of the experiment, the results shown in Table IV.

Table IV.

Age of cysts (in days)	18	20	22	24	26	28
Resulting culture	+	+	−	+	+	−

Most of the cysts inoculated into the last 5 cultures appeared to be dead: but that *some* were alive on the 24th and 26th day, the result clearly proves. Since an approximately equal inoculum was used for each culture, the negative

[1] The last day—the material being then exhausted.

experiment on the 22nd day shows, further, that the majority were really dead by that date, and the later positive results were due to the presence of isolated survivors.

The strains of amoebae so obtained were normal in every way: and that of the 24th day was continued for over 2 months (from 3. viii. 26 to 12. x. 26 —17 serial subcultures), and could have been maintained for an unlimited period.

The above experiment proves, therefore, that cysts of *E. histolytica*, passed in the faeces, can survive at room-temperature for at least 26 days. But this is certainly not the maximum time.

As explained elsewhere (Dobell and Laidlaw, 1926), it is usually easier to obtain cultures from cysts formed in cultures than from those passed in the faeces. With the latter, there is always a chance that some unfavourable bacterium may be introduced into the cultures, and so inhibit development of the amoebae. This source of fallacy can be eliminated by studying the survival of culture-cysts, and I have therefore made a long series of experiments with these to test their viability at room-temperatures. The results, during the first 4 weeks of experiment, have been generally similar to those just recorded, and therefore need not be described. Cultures can be obtained occasionally from culture-cysts kept for 4 weeks, but after this time they seldom develop. (I have tested them at various times up to the 50th day.) The last 19 experiments which I have made gave the results shown in Table V, which may be used in illustration of what has just been said.

Table V.

Age of cysts (in days)	28	29	30	31	32	33	35	37	39
Cultures	−	+	−	−	−	+	−	+	−
	+	−	−	−	−	.	.	.	.
	.	.	−	−	−	.	.	.	.
	.	.	.	.		.	.	.	.
	.	.	.	.	−	.	.	.	.

Each sign (+ or −) here denotes, as before, the result of a separate experiment. (For example, 5 cultures inoculated with cysts 32 days old were all negative; and of 2 cultures made from 29-day cysts, one was positive and one negative.)

All the positive cultures contained normal amoebae, which could be propagated further without difficulty. The strain derived from cysts kept for 33 days was maintained for 10 days before it was voluntarily abandoned; and during this time it again produced normal cysts which were also tested, and which hatched normally. The strain from 37-day cysts has been continued up to the time of writing (2 months), and has remained typical throughout. Under suitable conditions it continues to form normal cysts, which hatch normally and have been proved, by animal experiment, to be infective.

These results show, therefore, that the cysts of *E. histolytica* can survive

at room-temperature, under favourable conditions, for at least 37 days. That even this is the maximum time I cannot assert, for more protracted experiments may prove that it is too low a determination.

From numerous earlier observations I formerly concluded that the cysts of *E. histolytica*—from human faeces—"will survive for several weeks outside the body of man, if they are kept moist and cool"; and I noted that "the longest time of survival which I have observed is five weeks (cysts kept in water), but as a rule they will not live so long" (Dobell, 1919, p. 47). At that date it was not possible to check these microscopic observations by cultures; but I have now done so, and found that they are substantially correct[1]. (The difference between 5 weeks and 37 days is immaterial.)

Yorke and Adams (1926 *a*) quote—with apparent disapproval—the entire passage from which the foregoing extracts are cited. They themselves find that the cysts of *E. histolytica* can live for only 10 days in water at room-temperature. It is certain, however, that this is very far from being a correct estimate, and at present I see no reason for changing a single word in the passage which I wrote in 1919. It is certain that cysts of *E. histolytica* can and do live at ordinary laboratory temperatures, under otherwise favourable conditions, for at least 5 weeks—not for merely 10 days: and the presumption seems justified that they remain, in suitable circumstances, infective to man for a like period.

By repeated trials I have convinced myself that the methods used by Yorke and Adams are quite inadequate for determining the true maximum time of survival of cysts. In such experiments the bacterial content of cultures is of great importance: and when—as by their methods—it is entirely uncontrolled, negative results are often obtained because the cysts are not given a fair chance of developing. Moreover, the original medium of Boeck and Drbohlav is by no means the best for the cultivation of *E. histolytica*. All the results just described with cysts from cultures have been obtained with a constant and highly favourable bacterial flora present throughout, so that this factor has been controlled and invariable; and I have used methods of cultivation which are certainly, in many ways, superior to the original Boeck-Drbohlav technique. This may account, in part, for the considerable discrepancies between the findings of Yorke and Adams and my own.

It will be seen that my results, as a whole, indicate that the amoebae and the cysts of *E. histolytica* have inversely proportionate viabilities at body-temperature and at room-temperature. At 37° C. the amoebae can live for about 5 weeks, at 10–20° C. for about 3 days: whereas the cysts of this species can live for 5 weeks at room-temperature, but for not more than a few days at the temperature of the human body.

[1] Boeck (1921), using the eosin test of viability, found that cysts of *E. histolytica* might remain alive in distilled water for any time up to 153 days. (Only one cyst "survived" for this lengthy period.) He also found that they "lived" for even 211 days in sealed slide-preparations: but it is unlikely that he would have reached such conclusions if he had been able, at that date, to check them by the methods of cultivation which he subsequently discovered.

(3) *How long can the amoebae of* E. histolytica *survive exposure to dilute hydrochloric acid* (*N*/20 HCl)?

It has already been stated (Dobell and Laidlaw, 1926) that *E. histolytica* amoebae are rapidly killed by dilute hydrochloric acid (*ca.* 0·2 per cent.) at ordinary room-temperatures. Acid of this concentration usually kills most amoebae in a few minutes, though some individuals may survive longer; and from all our experiments we concluded that 30 minutes is the maximum time of survival. All my later observations confirm this conclusion.

The great difficulty here—as in the experiments previously described—is to obtain material containing amoebae *only*. Since cysts are far more resistant to acid than free amoebae, the presence of a single cyst, among the amoebae used for experimentation, may be sufficient to vitiate all results. In my earlier experiments this contingency was not rigidly excluded, and I have therefore repeated them under more stringent conditions. Methods of obtaining amoebae alone—unmixed with cysts—have already been discussed (p. 296 *seq.*), and the technique employed in this inquiry is essentially similar to that just described for studying the viability of amoebae at room-temperature. Cultures containing amoebae (unmixed with cysts) were treated with a large excess of dilute acid[1]; the solution neutralized at various intervals of time afterwards; and the amoebae then inoculated into favourable medium (previously sown with bacteria suitable for the strain of amoebae studied) and incubated and examined—the cultures, if negative, being subcultivated. By such methods it is possible to ascertain how long amoebae can withstand exposure to a given concentration of acid at a given temperature.

After many trials I have found HCl = *N*/20 (0·18 per cent.) the best for general purposes, and most of my later experiments have therefore been made with solutions of this strength. As a rule hydrochloric acid in this concentration kills *E. histolytica* amoebae in a few minutes at room-temperature. Isolated individuals may, however, survive for 20 minutes or possibly half an hour[2]. I have never recovered them in culture after a longer exposure. As an illustration I will give (Table VI) the combined results of my last two series of experiments, on which I place considerable reliance as they were performed under the most stringent conditions.

Table VI. *Culture-amoebae* (*no cysts*) *exposed to* *N*/20 HCl *at room-temperature.*

Time (minutes)	10	20	30	60	90
Culture	–	–	–	–	–
Subculture	–	+	–	–	–

[1] See Dobell and Laidlaw (1926), p. 306.

[2] Dobell and Laidlaw (1926), p. 306. It is possible that the positive result obtained in this experiment was really due to the accidental presence of cysts. With cultures known to contain amoebae *only*, I have been unable to repeat it.

I interpret a result such as this to mean that $N/20$ HCl kills all amoebae usually within 10 minutes, but that an individual organism may sometimes survive for as long as 20 minutes[1].

All my experiments, taken together, show that *E. histolytica* amoebae (all strains studied) cannot live longer than 30 minutes at room-temperature in $N/20$ HCl. In order to remove all amoebae, with absolute certainty, from a mixture of cysts and free forms, I therefore consider it necessary to treat it with acid of this concentration for over half an hour.

I have found, moreover, that individual amoebae of this species can survive exposure to $N/40$ HCl for at least 1 hour; while cysts have lived in $N/10$ HCl for as long as 3 hours at ordinary room-temperatures, and may possibly be able to live longer.

For the purpose of certain other researches which I wished to carry out, it was necessary to determine also how long *E. histolytica* amoebae can survive exposure to $N/20$ HCl at body-temperature. I therefore made a series of similar experiments in which the temperature was continuously maintained at 37° C. I imagined that, at the higher temperature, the acid would be more effective, and the amoebae would be killed more rapidly[2]. To my surprise I found that this is not so. The amoebae can withstand exposure to $N/20$ HCl at 37° C. for any time up to one full hour. As an illustration of my findings, I give, in Table VII, the combined results of my last 4 series of experiments.

Table VII. *Culture-amoebae (no cysts) exposed to* $N/20$ HCl *at* 37° C.

Time (minutes)	10	15	20	30	45	60	75
Cultures	+	+	+	+	+	+	−
	.	.	.	+	+	−	.

Each sign (+ or −) here denotes the final result of a separate experiment. (Negative primary cultures were invariably inoculated into subcultures, and in several instances this gave a positive final result.) In no experiments has a positive result ever been obtained finally from amoebae treated for longer than 60 minutes. Microscopic examination shows clearly, moreover, that the majority of individuals are killed much sooner. In the acid the amoebae at once become rounded and motionless, and most of them soon appear—and are—dead or dying. It is only by attempting to make cultures from them afterwards that one can determine whether such forms are really dead or alive.

[1] This experiment also illustrates the importance of making a subculture whenever a primary culture is negative. If this had not been done, in the present instance, all the results would have been negative: and the conclusion would have been drawn that all amoebae were killed after exposure for only 10 minutes. It is clear, however, that at least one organism actually survived for twice as long.

[2] If the problem were purely chemical, then it might be expected—following van 't Hoff's rule—that by raising the temperature about 20° C. (from 17° C. to 37° C.) the acid would act 4 times as effectively, and the amoebae would therefore all be killed at body-temperature in about a quarter of the time observed at room-temperature.

I am satisfied by these (and many other) experiments that *E. histolytica* amoebae can withstand exposure to *N*/20 HCl at 37° C. for a much longer time (1 hour) than they can at room-temperature (20 minutes). The explanation of this is, doubtless, that cooling, in itself, is harmful to them; while at 37° C.—their optimum temperature—their powers of resistance are at a maximum.

It follows from these findings that if one desires to make cultures from *cysts* of *E. histolytica*, and uses for this purpose material containing (or which possibly may contain) amoebae also, then the mixture must be treated with *N*/20 HCl *for more than* 1 *hour* to kill all free forms with certainty[1] if the experiment is made at body-temperature.

(4) *Must the cysts of* E. histolytica *be cooled before they can hatch?*

When *E. histolytica* encysts in cultures, the cysts, when fully formed, usually do not hatch forthwith but gradually degenerate and die on continued incubation at 37° C.[2]. I have seen this happen so often that I cannot doubt the correctness of the observation. If, however, the cysts are allowed to cool to room-temperature—at which they can live, as has been shown, for many days—they are then able to continue their development and hatch when transferred to suitable fresh medium and again incubated at body-temperature. This appears to indicate that ripe cysts require to be cooled before they can develop further—in agreement with what must normally occur in the life-cycle of *E. histolytica* in nature.

The following experiment, to which, at the time (February, 1926), I attached considerable importance, seemed to confirm this supposition.

Freshly passed faeces from a monkey (*M. sinicus*)—containing abundant cysts of *E. histolytica*—were at once treated with excess of dilute HCl solution (0·2 per cent.), and the cysts concentrated by sedimentation. The acid was allowed to act for 1¼ hours at room-temperature (in order to kill all amoebae), and was then neutralized. The cysts were then left to settle, and inoculated at various intervals of time into culture-tubes (previously sown with suitable bacteria[3]) and incubated, negative primary cultures being subcultivated. The result of the complete experiment is shown in Table VIII.

Table VIII.

Age of cysts (in hours) from time when they were passed	1¼	6	24	31	48	72	96
Culture	−	−	−	+	+	+	+
Subculture	−	−	+	.	.	.	.

This result appeared to show that the cysts were not able to develop further until they had been kept cool for about a day; and as various other

[1] Cysts of *E. histolytica* can survive exposure to *N*/20 HCl for at least 2 hours at 37° C.—as I have determined by experiment.

[2] Dobell and Laidlaw (1926), p. 304.

[3] "*Bacillus* I"—Dobell and Laidlaw (1926), p. 309.

experiments and observations led to the same conclusion[1], Laidlaw and I stated[2] that cooling is necessary in order that the cysts can continue their development. It is implied—though not definitely stated—in the paper by Yorke and Adams (1926), however, that freshly-passed cysts are able to hatch without previous cooling; and although these workers give no convincing experiments in support of this supposition, it appeared to me desirable to investigate the matter further. The question is important, but not easy to answer. It obviously cannot be answered by merely taking cysts from stools or cultures and incubating them, without cooling, in fresh medium: for as free amoebae are, or may be, carried over with the cysts by any such procedure, positive cultures could easily be thus obtained even though every cyst failed to develop.

I tried to solve the problem by experimenting with cysts derived both from cultures and from faeces. In order to make certain that no cooling could occur at any stage, I conducted every operation in the hot-room at the National Institute, which is kept at a constant temperature of 37° C. Everything used in the experiments (microscope, glassware, solutions, etc.) was previously adjusted to the same temperature, so that no variation in this factor was possible at any stage[3].

(1) *Cysts from Cultures.*—A culture of *E. histolytica* (Strain *K.* 28 *C.*, from *M. sinicus*) was incubated at 37° C. until it was found to contain abundant ripe cysts. These cysts were then treated with a large excess of *N*/20 HCl for 75 minutes, in order to kill all accompanying amoebae with certainty[4]; the solution was then neutralized with $NaHCO_3$, and left standing for 20 minutes—when most of the cysts had settled to the bottom of the test-tube. They were then pipetted into a tube of suitable culture-medium (previously inoculated with a pure culture of bacteria favourable[5] for the growth of the strain used) and incubated, and examined on the following day. The result was positive—a very good culture of amoebae being obtained. This strain was continued by subcultivation for 8 days—in order to make sure that it was healthy—and during this time typical cysts were again obtained

[1] Not only is this conclusion wrong—as will be shown presently—but the experiment (Table VIII) itself was fallacious: for on repeating it later, I obtained an excellent primary culture from cysts only 5 hours old.—A possible explanation of the results given above is that some harmful micro-organism or substance (from the faeces) may have been unwittingly introduced with the inoculum into the first two cultures in the series. Moreover, as sedimentation of the cysts was not complete when these tubes were inoculated, they probably received fewer cysts than the remainder; and they also showed more *Blastocystis*, which may have suppressed the amoebae. I can think of no other plausible explanations. The experiment was made with great care, and the observations were, I feel sure, correct.

[2] Dobell and Laidlaw (1926), p. 304.

[3] The usual bacteriological precautions to avoid contamination of the cultures—which might vitiate the results—were, of course, also observed in these and all other experiments.

[4] See preceding section, III (3). Microscopic examination showed that this drastic treatment killed not only the amoebae but many of the cysts as well.

[5] "*Bacillus* I"—Dobell and Laidlaw (1926).

from it, and stained preparations made and examined. The amoebae were in every way normal, and could have been propagated *ad libitum*.

A sample of the original cysts used in this experiment was kept and cooled, and not treated with acid. These cysts were tested culturally after keeping at room-temperature for 16 days, and again after 28 days. The result was positive in both experiments. (This was done in order to make sure that the cysts were normal and viable—in case the experiment at 37° C. had proved negative.)

I repeated this experiment with another strain of *E. histolytica* and slight variation in technique, and obtained an identical result.

(2) *Cysts from Faeces.*—In order to obtain uncooled cysts from faeces, I took one of my monkeys (*M. sinicus*) into the hot-room (37° C.). As soon as he defaecated, I examined his faeces microscopically and found that they contained—as anticipated—many cysts of *E. histolytica* and a few amoebae. This faecal material was forthwith emulsified in sterile distilled water, the larger particles were strained off, and the suspension of cysts was then treated with acid to kill the amoebae—the technique subsequently employed being similar to that just described for culture-cysts. After neutralization, the cysts were inoculated into suitable culture-medium (previously sown with *Bacillus* I) after various intervals of time. On incubating and examining these cultures —and when necessary making subcultures—I finally obtained the results shown in Table IX.

Table IX.

Age of cysts (in hours) from time when passed	1½	6½	11	24
Culture	+	−	−	−
Subculture	.	+	+	+

All the results were ultimately, therefore, positive. The material used for the first culture was relatively poor in cysts—though it gave the only positive primary culture—because they had not had time to sediment completely in so short a period. In the material used for the last two cultures, moreover, the cysts appeared, on microscopic examination, to be mostly dead. The majority had undoubtedly degenerated after standing in neutral water (with faecal particles) for 11 hours at 37° C. There was no evidence of hatching under these conditions.

The first culture (1½ hours) was examined microscopically during the early stages of its development, and normal excystation was actually observed to take place in it. The other cultures contained too few living cysts for such observations to be practicable.

As I was fully satisfied with the positive results obtained in this experiment—a somewhat trying one to make—I did not repeat it.

From the foregoing experiments, in which all possibility of cooling below 37° C. at any stage was rigidly excluded, and which were performed with great care (after making numerous preliminary experiments in order to define

and eliminate the chief sources of error), I therefore obtained consistent results which supply a conclusive answer to the question asked. It is certain that, under otherwise suitable conditions, the ripe cysts of *E. histolytica* are able to hatch—and the resultant amoebae to continue their development—if kept continuously at a temperature of 37° C. from the moment when they attain maturity.

(5) *Are the cysts of* E. histolytica *capable of further development as soon as they are fully formed?*

It was previously stated (Dobell and Laidlaw, 1926) that cysts, when fully formed, are not able to develop further unless they are cooled and kept for a certain time. That cooling is unnecessary I have just shown; and the experiments quoted also show that keeping, for any considerable time, is equally unessential. Cysts can continue their development, under certain conditions, immediately after they have reached maturity. I will add a few further notes bearing on this subject.

When a batch of cysts of *E. histolytica* (however obtained—whether from faeces or in a culture) is examined, the individuals are usually found in many different stages. Some are uninucleate, others quadrinucleate (mature), and others in various intermediate conditions. Among the quadrinucleates, however, great differences are often observable. Some contain abundant glycogen and chromatoid bodies, others practically none; while others show transitional stages. It seems reasonable to suppose that glycogen and chromatoids are reserve substances (carbohydrate and protein respectively) which the amoebae store in their cysts to enable them to live outside the body, when they are no longer free and able to capture food. If the cysts are kept under observation, both glycogen and chromatoids gradually disappear—rapidly at 37° C. and slowly at room-temperatures, as I have repeatedly observed. When a cyst reaches its mature (quadrinucleate) state, it is unable to ingest food and therefore draws upon its reserves. If it remains in the body, it uses up its glycogen and chromatoids quickly; but if it leaves the body, when just mature, its stored-up food will probably enable it to survive for a considerable time—according to its quantity. It thus appears probable that the amount of reserve material present in a cyst determines not only the time for which it can survive, but also the rapidity with which it hatches[1] when placed in a suitable environment.

From these considerations—supported by many observations and experiments—I am led to believe that quadrinucleate cysts of *E. histolytica*, when they contain abundant reserve material, can live for a long time, but can hatch but slowly: whereas cysts containing little or no reserves are ready to hatch quickly, but can live for only a relatively short period.

[1] I have found that cysts do not normally hatch until all their glycogen and chromatoids have disappeared, and other workers have apparently made similar observations.

Whether this be a legitimate inference or not, it is certain that cysts differ greatly in their powers of survival and in their times of hatching. Most cysts live (at room-temperature) for not more than 2 or 3 weeks, though some can survive for more than 5—as already shown. And when cysts are incubated at 37° C. in suitable medium, some may hatch after only $2\frac{1}{2}$ hours, while others[1] may require at least $17\frac{1}{2}$. Hatching may take place also at any intermediate time. Of this I have had ocular demonstration.

The experiment with a monkey—described on p. 306—showed that cooling the cysts is unnecessary: but it also showed that they are able to continue their development almost immediately after leaving the body. In this experiment the cysts, after treatment with acid at 37° C., were immediately incubated in culture-medium at the same temperature; and excystation was actually observed in the first culture after $5\frac{1}{2}$ hours' incubation (approximately 7 hours after the cysts left the body)[2].

It thus seems certain that the cysts of *E. histolytica* are capable of immediate further development as soon as they are mature; though the time taken in maturation, and the rapidity with which hatching may occur, depend upon various internal and external conditions (temperature, amount of reserve material present, etc.).

It also appears legitimate to conclude, from the observations just mentioned and others previously recorded, that ripe cysts of *E. histolytica*, if swallowed, may be infective—under favourable conditions—from the moment when they leave the body[3] and for a period of at least 5 weeks thereafter. The experimental foundations for such a conclusion are, if not secure, at least very strong.

(6) *Can encystation be immediately followed by excystation without change of environment?*

This is an important question, and one not easy to answer. On general grounds it is improbable that the cysts of *E. histolytica*, when formed in the bowel, usually develop further (*i.e.* hatch): for the function of the cyst—*pace* Hegner (1926)—is obviously to carry the parasite from host to host. Cysts are adapted to a life outside the body, amoebae to a life inside it. Analogies too numerous to mention must occur to everybody who reflects on the subject; and I therefore felt justified in writing six years ago that "it is certain that

[1] There is also cultural evidence (see p. 296) that some cysts may require incubation for as long as 24 hours or more before they can hatch.

[2] The exact times were as follows: cysts passed 10.40 a.m. (24. ix. 1926); inoculated into culture (after treatment with acid) at 12.5 p.m.; first amoeba seen emerging from its cyst 5.20 to 5.30 p.m.

[3] Up to the present my earliest successful infection experiment (with a kitten) has been obtained with cysts—freed from concomitant amoebae by treatment with acid—which were 5 hours old (*i.e.* 5 hours after leaving the body).

the cysts never hatch in the colon, where they are formed, or outside the body[1]."

Since I wrote this it has been shown, beyond all reasonable doubt, by Sellards and Theiler (1924) that kittens can be experimentally infected with *E. histolytica* by means of cysts (from human faeces) introduced into the large intestine. Yorke and Adams, and others, apparently consider that this observation contradicts my statement: but it is surely unjustifiable to assume that because excystation may occur in the large intestine of the kitten (where encystation, so far as is yet known, never occurs), it must therefore also take place in the human colon—where only encystation is known to take place. I do not doubt that the observations of Sellards and Theiler are correct[2], but their bearing upon the normal life-history of *E. histolytica* is by no means obvious. They certainly do not prove that encystation and excystation can take place in an identical environment, or that cysts can hatch immediately in the colon in which they were formed.

I have attempted to obtain light on this question by experiments *in vitro*. When *E. histolytica* encysts in cultures, the fate of the cysts can be studied; and it is possible to see whether, when they have completed their development, they hatch on continued incubation. As has already been recorded (Dobell and Laidlaw, 1926), cysts are generally formed, *in vitro*, towards the end of the life of the culture. In a young culture the amoebae multiply rapidly; later some of them, under suitable conditions, encyst; and finally, on continued incubation at 37° C., the cysts do not hatch but degenerate and die. If this be regarded as a parallel to what normally happens in the body, then it must be assumed that the cysts, when they reach maturity in man, likewise do not hatch, but, if retained and incubated in the colon for sufficient time, ultimately perish.

These observations, however, do not really answer the question at issue. A developing culture undergoes continuous change, and its composition varies from day to day. Generally speaking, it becomes progressively less suitable for the maintenance of its amoebic population; and after a certain time it becomes an unfavourable environment not only for cysts, but also for un-

[1] Dobell and O'Connor (1921), p. 25. It is clear, I think, from the context, that "outside the body" here means "outside the human body under natural conditions." Yorke and Adams (1926, p. 280) however—writing six months after I had myself announced that I had been able to hatch the cysts in cultures—quote these words with the apparent object of showing their error: and by changing the word "formed" into "found" they make my statement absurd—an absurdity unfortunately broadcast by the reviewer in the *Tropical Diseases Bulletin* (24, p. 15. 1927).

[2] I have not repeated their experiments because—when made with all precautions necessary—they are (to my mind) very cruel. The recorded results appear to me conclusive, and confirmation of them has already been supplied by Boeck and Drbohlav and by Hoare (1926). As regards the latter's single experiment, however, I feel some doubt. It was made by a far less rigorous method, and it is by no means certain (as he himself realized) that amoebae were not accidentally introduced with the cysts. My own findings show that this is possible—if not probable—when the technique described by Hoare is adopted.

encysted organisms. Consequently, in old[1] cultures both cysts and amoebae die on continued incubation. It is not surprising, therefore, that cysts should die, or fail to hatch, in medium which has become demonstrably unfavourable for the existence of the free forms.

It occurred to me that a fair test of a cyst's ability to hatch in the culture in which it was formed could only be made if the cyst were formed in a comparatively young culture—before the medium was exhausted. With most encysting strains of *E. histolytica* which I have hitherto studied, cysts have been formed late in the life of the culture; but occasionally they have appeared, in small numbers, abnormally early. For example, my Strain *D* of *E. histolytica* (from man) and Strain *J* (from *M. rhesus*) usually produced cysts[2] in greatest numbers on the fourth day of incubation, though the unencysted amoebae might live for a week or so in the medium used. But very rarely a few cysts appeared in such cultures as early as the second day, when the medium was undoubtedly still favourable for continued growth. I therefore determined to study such early cysts, if possible, but for long was unable to obtain them in numbers sufficient for any satisfactory experiments. I finally succeeded with another strain of *E. histolytica* (Strain *BC*, from *M. sinicus*). This strain grew luxuriantly, the cultures living usually for about 6 days[3]. For a time encystation occurred freely, the maximum number of cysts being generally obtained on the fourth day of incubation. On one occasion, however, cysts were formed prematurely in small numbers, among numerous amoebae, on the second day: and this culture was therefore removed from the incubator and cooled to room-temperature as soon as the cysts were detected. It was then kept at this temperature for 3 days, in order to kill all free amoebae. A small amount of the sediment in it was then inoculated into fresh medium (to see whether the cysts were viable in the ordinary way); and both the original culture and the subculture were then put in the incubator at 37° C. Both produced a crop of active normal amoebae after 24 hours' incubation. I concluded, therefore, from the original (reincubated) culture, that the cysts actually hatched, after cooling, in the medium in which they were formed.

A second similar culture containing premature cysts was kept at room-temperature for 4 days—to eliminate any possibility of the previous result having been due to the presence of free amoebae which had survived cooling for 3 days. The result, however, was the same.

These two experiments appeared to me conclusive: nevertheless, I have repeated them with another strain of amoebae. By good fortune I have now a strain which makes this easy. Strain *K*. 28 *C*.—already frequently referred

[1] "Old" is, of course, a relative term. With some strains, in certain media, the culture may reach the end of its life in 2 days. With other strains, and other media, a similar stage may not be reached before a week or 10 days.

[2] See Dobell and Laidlaw (1926), p. 303 *et alibi*.

[3] In medium composed of inspissated horse-serum with Ringer-eggwhite and starch. See Dobell and Laidlaw (1926).

to—has this peculiarity: on cultivation by a certain method it invariably produces cysts in large numbers on the second day of incubation; but the amoebae can always live, when cultivated in the same medium, for at least 4 or 5 days. (The medium in which cysts are formed is therefore not unfavourable for continued cultivation.) If a culture containing cysts of this strain is removed from the incubator and kept at room-temperature, it will produce a new crop of amoebae—derived from the cysts—on reincubation at any time up to the ninth day[1]. As it is certain that no amoebae of this species can survive for 9 days at room-temperature[2], it follows that the cysts of this strain of *E. histolytica* can hatch in the same medium as that in which they were formed.

In an experiment of this sort, the cysts are obviously not merely kept until they hatch in the medium in which they were formed: they are also subjected to cooling (to kill accompanying amoebae) and their time of hatching is artificially delayed[3]. The environment also undergoes *some* change in the process; and the reincubated medium is not, therefore, exactly the same as that in which encystation took place. I have already shown[4], however, that cysts *can* hatch when kept continuously at 37° C. from the time when they are fully formed: and the changes undergone by the medium are probably trivial, and not such as would materially influence the result of the experiment.

Although excystation can thus occur in reincubated cultures, under artificial conditions, it does not follow that cysts normally hatch in the colon in which they are formed. Encystation probably occurs normally in the lumen of the large gut and the cysts are carried passively along with the faeces, which gradually become dense and formed as they approach the anus. It is improbable that excystation would occur in solid faeces: and no trustworthy worker has ever yet—so far as I am aware—found hatching cysts in freshly passed stools.

It seems to me probable that when the cysts are fully formed in the bowel they behave as they do usually in cultures. They degenerate and die on prolonged incubation—whether in the colon or in the test-tube. But this is an inference which requires further verification. I cannot doubt, however, after making the foregoing experiments, that cysts *may* hatch, under peculiar conditions, in the colon in which they were formed. If a violent antiperistalsis were to carry them from the lower end of the large bowel towards the caecum, and if they were then retained in the fluid gut-contents hereabouts for some hours, it seems very probable that excystation might occur. It also seems likely that the experiment of Sellards and Theiler might succeed if made on man. But in the absence of concrete evidence it would be idle to speculate further on such matters.

[1] Possibly even later; but I have not yet tested it beyond this date.

[2] See preceding section III (1).

[3] This applies also, of course, to the experiment of Sellards and Theiler, and is a point which does not appear to have been considered by them or by other workers.

[4] See preceding section III (4).

IV. Conclusions.

(1) Under artificial (cultural) conditions, the free amoebae of *E. histolytica* —both human and simian strains—may survive at ordinary room-temperatures for any length of time up to 3 days; but there is no evidence that they can ever live longer. At 37° C., however, under similar artificial conditions, some individuals may survive for at least 5 weeks.

(2) The cysts of *E. histolytica* can remain alive at room-temperature for any time up to at least 37 days, though they usually die within a few days if kept continuously at body-temperature.

(3) At room-temperatures the amoebae of *E. histolytica* are usually killed by exposure to *N*/20 HCl for only a few minutes. Isolated individuals, however, may withstand such treatment longer (up to 20 minutes), though none survive for more than half an hour. But at 37° C. individual amoebae may survive exposure to acid of this concentration for any time up to one full hour, though the majority are killed much sooner.

(4) The cysts of *E. histolytica* can continue their development (*i.e.* can hatch, and produce normal, viable, and readily cultivable amoebae) if kept continuously at 37° C. from the moment when they reach maturity. Previous cooling is not necessary to induce excystation: and under artificial conditions the cysts are able to continue their development immediately—without any period of rest intervening.

(5) Encystation and excystation can take place in the same surroundings. Under cultural conditions the cysts of *E. histolytica* can hatch (and the resultant amoebae can develop) in the same environment as that in which they were formed.

The foregoing conclusions are drawn directly from observations and experiments. My further inference from them all—which is less securely founded, and is largely an expression of personal opinion—is that these findings should be taken into consideration by all who attempt to cultivate *E. histolytica* or other entozoic amoebae from the encysted forms. They should also, I think, be remembered whenever the life-history of *E. histolytica* is, in future, discussed; though I would emphasize the necessity of exercising the greatest caution when translating cultural experiments and observations into terms of normal and natural development.

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REPORT ON A COLLECTION OF CESTODA, MAINLY FROM EGYPT.

PART I. FAMILIES ANOPLOCEPHALIDAE, DAVAINEIDAE.

By F. J. MEGGITT.

Zoology Department, University of Rangoon.

(With 6 Text-figures.)

Introduction.

The present paper is the first of a series describing a collection of cestodes from the Biological Laboratory of the Egyptian Government School of Medicine, Cairo, the nematodes having previously been described in this *Journal* by Baylis (1923 *a*, 1923 *b*, 1923 *c*) and Boulenger (1926). The identification of this portion of the collection has presented great difficulties, the specimens, from long immersion in alcohol mixed with fragments of cork, having become so pigmented as to be virtually black and almost unstainable: but for a formula (soak for 3 to 5 days in pure beechwood creosote, then transfer for the same time to 5 per cent. hydrochloric acid in 70 per cent. alcohol) supplied by Dr H. A. Baylis—to whom the author tenders his most sincere thanks—the elucidation of their anatomy would have been impossible. In many tubes the tapeworms were present only in fragments and often several species were mixed together. While great care has been taken to examine every particle that has varied even slightly in general appearance from the remainder, and a sufficiently representative quantity of the tube taken in every case yet, considering that differences between genera are only rarely observable without staining, it is obvious that, in the first place in a tube of several hundred fragments all alike it is impossible to exclude the possibility of overlooking a stray species and, in the second, when sorting the strobilae it is equally impossible to ensure that no mistake has been made and a few fragments of one species placed with another.

In conclusion the author wishes to express his indebtedness to Prof. G. H. F. Nuttall for having entrusted him with so valuable a collection of material. The specimens are in the collection of the Molteno Institute for Parasitology, Cambridge, under the numbers preceding each species.

Family ANOPLOCEPHALIDAE Fuhrmann, 1907.

Sub-family Anoplocephalinae Blanchard, 1891.

Bertiella Stiles and Hassall, 1912.

From this genus *B. omphalodes* (Hermann, 1783) was separated by Lühe (1910) as the type of a new genus *Paranoplocephala* characterised by alter-

nating genital pores and the presence of an external vesicula seminalis: this classification has recently been followed by Baer (1925, p. 20). The characters in question hardly seem of generic rank, in particular the alternation of the genital pore which, in *Bertiella*, is not invariably regular. The occurrence of two species from widely separated areas, in the absence of important morphological characters, does not appear of much importance. It therefore seems advisable to consider *Paranoplocephala* as a synonym of *Bertiella*.

825. **B. fallax** n.sp. (Fig. 3).

Host. *Cebus capuchinus* (L. 1766).

Locality. Egypt.

Length 175[1], breadth 5. Scolex 0·5 dia., devoid of pigment. Genital pores irregularly alternate, with a tendency to be unilateral, in anterior half of proglottis margin. Genital cloaca with an external narrow portion followed by an inner globular part, into the extreme inner end of which open the genital ducts. Cirrus sac 0·26 long, extending to or just past longitudinal excretory vessel. Vas deferens only slightly coiled. Testes approximately 200, in two groups, separated from the female glands but joined by one or more rows along posterior margin of proglottis; the poral group is entirely posterior to the genital ducts, but the aporal extends to the anterior margin. Vagina dilated as far as poral edge of ovary, then suddenly contracted to a thin tube. Ovary median, crescentic, with numerous outwardly directed finger-like lobes. Vitelline gland posterior to ovary. Eggs without pyriform apparatus.

372, 667. **B. mucronata** (Meyner, 1895).

Host. *Cercopithecus* sp. (372); *Mycetes niger* (667).

Locality. London; Egypt.

On studying the contents of these two tubes, one labelled "Bertiella mucronata from Mycetes niger," the other "Bertiella cercopitheci from Cercopithecus sp."—the latter a co-type?—no real difference was found. *B. cercopitheci* is therefore regarded as a synonym of *B. mucronata*. The differences between these two species, *B. conferta* (Meyner, 1895), *B. polyorchis* (Linstow, 1905) and *B. studeri* (Blanchard, 1891) appear but slight and such as might be produced by variations in methods of fixation or degree of muscular contraction of the worm; it is probable that all these are but varieties of one species.

Killigrewia n.g.

A single set of genital organs in each proglottis. Genital pore irregularly alternates. Genital ducts pass dorsal to longitudinal excretory vessels. Testes in two approximately equal groups, completely separated from each other by the female glands. Ovary slightly poral. Uterus a transverse sac with anterior and posterior diverticulae. Adults in birds and mammals. Type-species *K. frivola* n.sp.

[1] All measurements in mm.

658 *g*. **K. frivola** n.sp. (Fig. 1).

Host. Label illegible.

Locality. Egypt.

Length 170, breadth 5. Scolex absent. All proglottides much broader than long. Genital pore irregularly alternates, at anterior quarter of proglottis margin. Genital cloaca small and tubular. Genital ducts pass dorsal to longitudinal excretory vessels. Cirrus sac 0·22–0·25 × 0·066–0·11 in mature segments, extending to or not quite to excretory vessels, containing a few coils of the vas deferens, a small vesicula seminalis, and one or two bends of the cirrus surrounded by prostate glands. Testes 103–145, in two entirely separate groups, one each side of female organs, 53–78 aporal, 59–68 poral, usually more poral than aporal. Ovary slightly poral, alternating from side to side of central line according to position of genital pore, bilobed, each lobe being subdivided into long finger-like transverse processes. Vitelline gland posterior to ovary, slightly lobed. Receptaculum seminis large. Uterus at first a narrow transverse tube, extending from ovary to posterior and outer margin of proglottis, later wider, with dorsal and ventral outgrowths tightly packed together, filling the whole proglottis. Eggs 0·033 × 0·025, without pyriform apparatus.

The present form shows greater affinity with the Anoplocephalinae than with any other sub-family. The single set of genital organs and the alternating genital pore separates it from all other genera in that sub-family except *Bertiella* Stiles and Hassall, 1912, *Andrya* Railliet, 1893, *Parabertiella* Nybelin, 1916, and *Triuterina* Fuhrmann, 1921. The position of the testes separates it from the third and the first, and the absence of a prostatic gland from the second. From the last it differs in the absence of an anterior row of testes connecting the two groups, in the absence of an aporal aggregation, in the absence of a third and anterior division of the uterus and in the course of the genital ducts. It is therefore necessary to create for it a new genus for which the name and diagnosis already given is proposed. To it belong the species already described, the one following, and *Bertiella aberrata* Nybelin 1916.

684. **K. pamelae** n.sp. (Fig. 2).

Host. *Turturoena sharpi*.

Locality. Egypt.

Length 70, breadth 1·7. Scolex 0·816 dia. Genital pore at anterior third of proglottis margin. Cirrus sac 0·18 × 0·06, extending to or just past longitudinal excretory vessels. Testes in two completely separated groups, approximately 37 poral and 28 aporal; centre of proglottis completely free from testes. Cirrus armed. Ovary crescentic rather poral. Receptaculum seminis large and spherical.

Moniezia Blanchard, 1891.

This genus has been the subject of a recent paper by Theiler (1924, p. 109) who has concluded, after a critical examination of the evidence, that of all

the numerous species ascribed to this genus only three (*M. expansa* Rudolphi, 1802, *M. planissima* Stiles and Hassall, 1893, and *M. trigonophora* Stiles and Hassall, 1893) are valid, with the possibility of a fourth (*M. alba* Perroncito, 1878); in several specimens of the latter he found interproglottidal glands present and from that concluded that this species might possibly be material that through age or degeneration had lost its staining power. While reserving a decision upon the specific value of variations of the interproglottidal glands, the conclusions of the author appear well founded and in the present paper are recognised only the four species listed by him.

366, 666, 831. **M. alba** (Perroncito, 1878).

Host. *Lama vicugna* (Molina, 1872) (366); sheep (666); ?damostictile (831).
Locality. South America (366); Egypt (666); Rangoon (831).
Placed in this species by reason of the absence of interproglottidal glands.

788, 830. **M. expansa** (Rudolphi, 1802).

Host. Unidentified ruminant (788); *Capra hircus* (830).
Locality. Egypt (788); Rangoon (830).
Goats and sheep in Rangoon are heavily infected with this parasite; it is hoped later to examine thoroughly this form.

Multitesticulata n.g.

Genital ducts pass to longitudinal excretory vessels. A single set of reproductive organs in each proglottis. Genital pore irregularly alternates. Cirrus sac large, cirrus armed. Testes completely filling proglottis, entirely surrounding female glands. Vaginal pore anterior to cirrus sac. Uterus primarily sac-like, secondarily a mass of irregular lacunae. Eggs without pyriform apparatus. Adults in Insectivora. Type-species *M. aegyptica*.

761. **M. aegyptica** n.sp. (Fig. 6).

Host. *Talpa europaea*.
Locality. Erythrea.
Length 60, breadth 0·56. Scolex absent. The small size of the dorsal longitudinal excretory vessel and the poor preservation of the material rendered it impossible for the course of the genital ducts to be ascertained. Genital pore irregularly alternates, almost at extreme anterior angle of proglottis. Genital cloaca fairly deep, extending nearly to excretory vessels; genital papilla present or absent according to state of protusion of cirrus. Cirrus sac 0·16–0·17 × 0·071–0·079 in mature proglottides, 0·23 × 0·076 in gravid ones, extending nearly to centre line of proglottis. Cirrus armed and coiled inside cirrus sac; latter also containing a few coils of the vas deferens. Testes 34–40, entirely surrounding female glands and completely filling all available space in proglottis, tightly packed together, persisting up to and past uterine stage. Vaginal pore anterior to cirrus sac, vagina crossing over

outer end of latter to open into a large receptaculum seminis, usually choked with spermatozoa, extending obliquely internally to centre line of proglottis; maximum size of receptaculum seminis 0·31 × 0·13 (cirrus sac same segment 0·23 × 0·076). Ovary central, horse-shoe shaped with concavity directed anteriorly. Vitelline gland dorsal to posterior end of ovary. Uterus persistent; at first sac-like, later a mass of irregular lacunae. Eggs without pyriform apparatus.

The only sub-families in which the present form can be placed are the Anoplocephalinae, Ophyrocotylinae and Dilepininae. The structure of the uterus removes the species from the second of these but can be paralleled by that of *Schizotaenia* in the first and *Choanotaenia* in the second; most mammalian cestodes belong to the first but the general disposition of the testes allies it with the second. The general appearance of the anterior extremity suggests the probability of the scolex being unarmed, in which case it certainly belongs to the Anoplocephalinae. In the absence of definite information the species is placed in that sub-family. As at present there exists no genus with such an assemblage of characters as that just described, it is necessary to create for the present form a new genus for which the name and diagnosis already given are suggested.

Sub-family Avitellininae Gough, 1911.

Avitellina Gough, 1911.

652. **A. centripunctata** (Rivolta, 1874).

Host. Sheep.
Locality. Egypt.

Sub-family Linstowinae Fuhrmann, 1907.

Inermicapsifer Janicki, 1910.

The work of Baer (1925, p. 30) has dissipated the confusion surrounding this genus and *Multicapsiferina* Fuhrmann, 1921 (synonyms *Arhynchotaenia* Pagenstecher, 1878; *Zschokkea* Fuhrmann, 1902; *Zschokkeella* Ransom, 1909); the conclusions arrived at in that paper are accepted and *Multicapsiferina* considered a synonym of *Inermicapsifer*.

648, 665. **I. hyracis** (Rudolphi, 1810).

Host. *Procavia abyssinicus*.
Locality. Gizah.
No label of host or locality in tube 665.

800. **I. interpositus** Janicki, 1910.

Host. *Procavia abyssinicus*.
Locality. Gizah.
Scolex 0·6 dia. Cirrus sac extending internally to longitudinal excretory vessels. Testes 80 or more, in a continuous double row along posterior border

of proglottis, extending on both sides laterally to excretory vessels, with a small poral and a larger aporal aggregation. Egg-capsules 64–75, each containing 5–9 eggs. Otherwise agreeing with the description of Janicki (1910, p. 385).

795. **I. otidis** n.sp. (Fig. 5).

Host. *Otis houbara*.

Locality. Egypt.

Length 100–200, breadth 4–5. Scolex 0·51–0·56 dia. Rostellum and hooks absent. Genital pore unilateral, at anterior third of proglottis margin; genital cloaca small, without sphincter. Cirrus sac 0·22 long, just reaching or not quite reaching longitudinal excretory vessels. Testes approximately 100, completely surrounding female glands except for the space occupied by the genital ducts, confined within the excretory canals. Female glands poral; ovary crescentic, with many lobes; vitelline gland closing the crescent and bilobed, each lobe being subdivided laterally. Eggs singly in thin-walled capsules, occupying the whole proglottis and not extending laterally to excretory vessels.

The only cestode with unilateral genital pores, unarmed scolex and eggs in egg-capsules recorded from birds is *I. linstowi* (Parona, 1885). The disposition of the testes and the size of the cirrus sac are sufficient to separate it from the present form.

Linstowia Zschokke, 1898.

This genus has tentatively been united with *Oochoristica* Lühe, 1898, by Baer (1925, p. 25), two species only (*L. echidnae* Thompson, 1892, *L. semoni* Zschokke, 1896) being reserved as a temporary measure. This arrangement appears to be based upon inaccurate evidence. "In both genera...the genital ducts may pass dorsally between or ventral of the excretory vessels"; an examination of the literature has shown that in the species usually attributed to *Linstowia* (*L. braziliensis* Janicki, 1904, *L. echidnae* Thompson, 1892, *L. jheringi* Zschokke, 1904, *L. semoni* Zschokke, 1896) the genital ducts pass ventrally to the excretory vessels, in *L. ameivae* Beddard, 1914, and *L. lemuris* Beddard, 1916, their course is not stated; in those attributed to *Oochoristica* the course is always dorsal or between. This difference appears sufficient to justify the separation into two genera. It is probable that *L. ameivae* should be removed to *Oochoristica*.

Oochoristica Lühe, 1898.

The species belonging to this genus exhibit great morphological similarity but equally great dissimilarity in hosts and distribution. For example, cestodes collected from *Calotes versicolor* in India (and made the basis of a new species —*O. sigmoides*—by Moghe, 1926, p. 52) agree in all but size with those of *O. crassiceps* Baylis, 1919, from a snake in German East Africa. Many of the existing species are distinguished solely by the absolute (as distinct from the

relative) size of various portions of the anatomy. In the opinion of the author this distinction is inadmissible. In forms so liable to changes of size by muscular contraction or expansion, to changes of form by variations in methods of fixation, to rely purely upon measurements is to invite confusion. Theiler (1924, p. 112) states: "I have been able to stretch a fragment of live worm to at least five times its original length, without damaging it"; in such an extension the sizes of the various portions of the body must alter considerably. Baylis (1919, Pl. XXI, figs. 6, 7) has shown the great alteration which may be produced by differences in methods of fixation or by age. The experience of the author (1926, pp. 233, 236) has shown that the size of the cirrus sac is equally unreliable. The sole absolute measurement upon which any reliance may be placed is that of the rostellar hooks, and that only with a due allowance for wide variation; other measurements are valuable only in so far as they support other characters.

774, 791, 803. **O. agamae** Baylis, 1919.

Host. *Dendropus* sp. (774); *Agama* sp. (791); *Cerastes vipera* (803).

Locality. Egypt.

The specimens from *Agama* sp. showed nothing worthy of notice, those from *Dendropus* were not well preserved. Those from *Cerastes vipera* were 80 long, 1 broad. Genital pore at anterior quarter of proglottis margin; genital cloaca shallow and muscular. Cirrus sac 0·14–0·2 × 0·27–0·4 in mature segments (0·25 × 0·027; 0·14 × 0·04; 0·145 × 0·031 in three adjacent proglottides), extending just past or not quite to the excretory vessels according to degree of expansion. Testes 35–40, extending laterally to vitelline gland. Egg-capsules thinly scattered throughout the proglottis, extending externally to excretory vessels. Joyeux (1923, p. 162) described from *Cerastes cornutus* in Algeria an unnamed species of this genus which differs from the present form in the smaller number of testes.

771, 824. **O. crassiceps** Baylis, 1920.

Host. *Stellio vulgaris* (771); ? *Gongylus* (824).

Locality. Egypt.

The specimens agree with the original descriptions except for the cirrus sac which, in the second tube, was 0·083–0·094 × 0·06–0·065 in mature proglottides and 0·098–0·12 × 0·046–0·054 in gravid ones, as compared with 0·09–0·1 × 0·03–0·04 in the first and 0·15 × 0·07 in the original specimens.

758. **O. fusca** n.sp.

Host. *Eryx jaculus*.

Locality. Sahara.

No complete strobilus present. Length of longest fragment 15, probable total length 30–40, breadth 0·54. Scolex 0·2 dia. Genital pore at limit of anterior quarter or fifth of proglottis margin. Genital cloaca and sphincter

poorly developed. Cirrus sac 0·07–0·1 long, extending internally to excretory vessels. Testes 30–38, posterior to ovary but lateral to vitelline gland. Egg-capsules numerous, tightly packed together, extending to margin of segment. The present form is similar to *O. agamae* Baylis, 1919, but differs in the number of testes and the development of the genital cloaca.

734. **O. truncata** (Krabbe, 1879).

Host. *Eryx jaculus*.

Locality. Sahara.

Cirrus sac 0·156–0·17 × 0·03–0·039 in mature proglottides, extending to or occasionally past longitudinal excretory vessels. Testes 32–38. Ovary much subdivided. Female glands rather poral. Otherwise agreeing with Rudin's description (1916, p. 75).

755. **O.** sp.

Host. *Stellio vulgaris*.

Locality. Egypt.

A few fragments in an exceedingly bad state of preservation, impossible to elucidate the anatomy. Genital pore irregularly alternates, anterior to centre of proglottis margin. Cirrus sac appears to extend to longitudinal excretory vessels. Eggs singly in capsules, scattered rather thinly throughout the whole proglottis.

765. **O.** sp.

Host. *Agama* sp.

Locality. Cairo.

Several immature fragments in a bad state of preservation. Genital pore at anterior third of proglottis margin. Cirrus sac reaching longitudinal excretory vessels. Testes 25–30, extending anteriorly to anterior level of ovary. Ovary in centre of proglottis. The only species of this genus with testes extending so far anteriorly are *O. zonuri* Baylis, 1919, and *O. megastoma* (Diesing, 1850). From the former the present form is distinguished by the fewer number of testes and the smaller size of the cirrus sac, from the latter again by the smaller size of the cirrus sac. The material is scanty and too badly preserved to suffice for the creation of a new species so that, as it differs by well-marked characters from all known species, it appears best to leave the present form unnamed.

Pancerina Fuhrmann, 1889.

Figures of this genus are not available in Rangoon. Its general anatomy (absence of a rostellum, isolation of eggs in parenchyma) place it rather with the Linstowinae than with the Dipylinae and in its reptilian host it agrees with *Oochoristica*. The diagnosis of the former family must therefore be altered to include forms with double genital organs.

750. **P.** sp.

Host. *Psammus arenaria*.

Locality. Cairo.

Sub-family THYSANOSOMINAE Fuhrmann, 1907.

Thysanosoma Diesing, 1835.

366 *a*. **T. giardi** (Rivolta, 1878).

Host. *Lama vicugna*.
Locality. South America.

Family DAVAINEIDAE Fuhrmann, 1907.

Sub-family DAVAINEINAE Braun, 1900.

Cotugnia Diamare, 1893.

772, ? 810. **C. digonopora** (Pasquale, 1890).

Host. Fowl.
Locality. Egypt.

The contents of these two tubes consisted of fragments in an exceedingly bad state of preservation. Tube 810 contained immature cestodes with a rostellum but without apparent hooks. Proglottides with double genital organs, testes approximately 60 in a band along posterior margin of segment, and a cirrus sac extending not quite to excretory vessels. In those in tube 772 nothing of the anatomy could be ascertained except the presence of double genital pores. In neither was the preservation sufficiently good to allow of sections being made, the material crumbling on cutting. The material is listed under this name by reason of the few resemblances it bears to the species but the characters given are not sufficient for a definite identification.

798 *a*. **C. fleari** n.sp. (Fig. 4).

Host. "Wandertaube" (Pigeon).
Locality. Egypt.

Length 45, breadth 4·5. Scolex 0·54–0·58 dia. Rostellar hooks 0·016–0·018 long, in two rows. Genital pore slightly anterior to centre of proglottis margin. Cirrus sac in mature segments 0·29–0·31 × 0·03, in young proglottides just passing longitudinal excretory vessels, in mature ones either extending to the vessels or just falling short. Testes in mature segments in two clearly separated groups; in each group 14–17 poral and 14–27 aporal, the ovary dividing each group into two. Egg-capsules numerous and rectangular by compression, often arranged regularly in longitudinal and transverse rows. I have great pleasure in naming this species after Captain Flear in acknowledgement of the help given in selecting specific names.

798 *b*. **C. cuneata** Meggitt, 1924.

Host. "Wandertaube."
Locality. Egypt.

In the same tube as the above species were others which differed greatly in external appearance and proved to be a separate species. The following table shows the differences between the two forms.

	C. cuneata	*C. fleari*
Length	10–21	45
Breadth	0·5	4·5
Number of testes	54–57	28–44
Arrangement of testes	One continuous band	Two separate groups
Cirrus sac	0·081 × 0·017	0·29–0·31 × 0·03

688. **C. polycantha** Fuhrmann, 1909 var. **paucimusculosa** nov.var.

Host. "Wandertaube."

Locality. Cairo.

The specimens in this tube agreed with Fuhrmann's description (1909, p. 120) with the exception of the musculature. This was very feebly developed, consisting of two layers each of transverse and longitudinal muscles, the latter composed of but few fibres and the outermost not extending to the subcuticula. The transverse muscles were still smaller, arranged in two cylindrical sheaths each with numerous irregular bosses, so that in transverse sections the layer appeared to be broken, a portion being pushed towards the exterior. This is in sharp contrast to the four layers of longitudinal and five of transverse muscles figured by Fuhrmann (1909, fig. 39). A similar variation in the musculature of two forms, otherwise identical, has already been recorded in the case of *C. cuneata* (Meggitt, 1924, p. 304).

658 *h*. **C.** sp.

Host. Label illegible.

Locality. Egypt.

A few fragments present. Greatest breadth 4. Scolex absent. Genital pore at centre of proglottis margin. Cirrus sac 0·17 × 0·03 in mature segments, extending nearly to excretory vessels. Testes 50–80, in a broad band across segment, external to excretory vessels, posterior and lateral to female glands, 4–5 deep antero-posteriorly at the widest part. Ovary touching excretory vessel. Egg-capsules too numerous to count, each containing a single egg and extending laterally to excretory vessels. The above characters are not sufficient to separate the present form from others of the same genus.

790 *c*. **C.** sp.

Host. *Platalea leucodia*.

Locality. Egypt.

A single macerated fragment consisting of a few mature segments. Genital pore in anterior half of margin. Cirrus sac 0·11–0·13 long, reaching longitudinal excretory vessels. Testes approximately 20, posterior, between and extending in a central straight line anterior to the ovaries, none directly anterior to the ovaries themselves, that space being occupied by the coils of the vas deferens. Ovaries much lobed, nearly touching each other, near excretory vessel. No cestodes with double reproductive organs have been recorded from the Ardeiformes. By reason of the inconclusive nature of the material the present identification can only be regarded as tentative.

Raillietina Fuhrmann, 1920.

681. **R. cryptacantha** (Fuhrmann, 1909).

Host. *Turtur sharpii*.

Locality. Egypt.

A single strobilus devoid of scolex. Genital pore unilateral, at centre of proglottis margin. Cirrus sac 0·058–0·065 × 0·09–0·11, reaching nerve. Testes 8–9 aporal, 2–3 poral; total 10–12. Female glands rather aporal. Egg-capsules approximately 100, each containing 3–5 eggs and, in posterior portions of proglottis, extending laterally to excretory vessels. Otherwise agreeing with Fuhrmann's description (1909, p. 105). The last character is important. The capsules lateral to the excretory vessels are few in number but appear to indicate that in more gravid proglottides they will increase in number.

65. **R. urogalli** (Modeer, 1790).

Host. ?

Locality. England.

658 *a*. **R.** sp.

Host. Label illegible.

Locality. Egypt.

Numerous fragments present. Greatest breadth 1·2. Scolex absent. Genital pore unilateral, at centre of proglottis margin; genital cloaca long and narrow. Cirrus sac in mature segments 0·12–0·15 × 0·05–0·06, extending to or not quite to excretory vessels. Testes 8–10 aporal, 4–5 poral. Egg-capsules 100–120 in each proglottis, each containing 7–9 eggs, and extending laterally to excretory vessels. In the absence of a scolex and a host name a definite identification is impossible.

Sub-family Idiogeninae Fuhrmann, 1907.

Idiogenes Krabbe, 1868.

703 *a*, 705. **I. flagellum** (Goeze, 1782).

Host. *Fulica cristata* (703 *a*); *Otis houbara* (705 *a*).

Locality. Egypt.

In tube 703 was a single specimen of this species; as all the remaining specimens belong to the genus *Hymenolepis* this record should be regarded with suspicion.

In tube 705 no scolex was present. The specimens agreed with Fuhrmann's description (1906, p. 81) with the exception that the aporal wing of the ovary was considerably larger than the poral and that the cirrus sac was curved, reaching the central or poral side of the anterior margin, not straight and reaching the aporal anterior corner. The testes appeared and disappeared quickly, four proglottides containing all the stages. *I. otidis* (Krabbe, 1869) is the only species previously recorded from Charadriiformes and is distinguished from the present one by the greater number of testes (30 instead of 10–12) and by the unlobed ovary.

Species of the genus **Idiogenes** *Krabbe*, 1868.

Species	No. of rostellar hooks	Size of rostellar hooks	No. of testes	Size of cirrus sac mm. long	Position of vitelline gland	Vagina
I. flagellum (Goeze, 1782)	130–150	10μ	10–12	0·24	Median	Coiled
I. grandiporus Cholodkovsky, 1905	104	28–34μ	30	—	„	—
I. horridus Fuhrmann, 1909	160	10μ	7–9	0·2	Poral	Coiled
I. horridus africanus Hungerbühler, 1910	160	—	—	—	—	Not coiled
I. nana Fuhrmann, 1925	75–80	7·2–9μ	9–11	—	—	Coiled
I. otidis Krabbe, 1869	—	24–26μ	10–15	0·15	Median	„

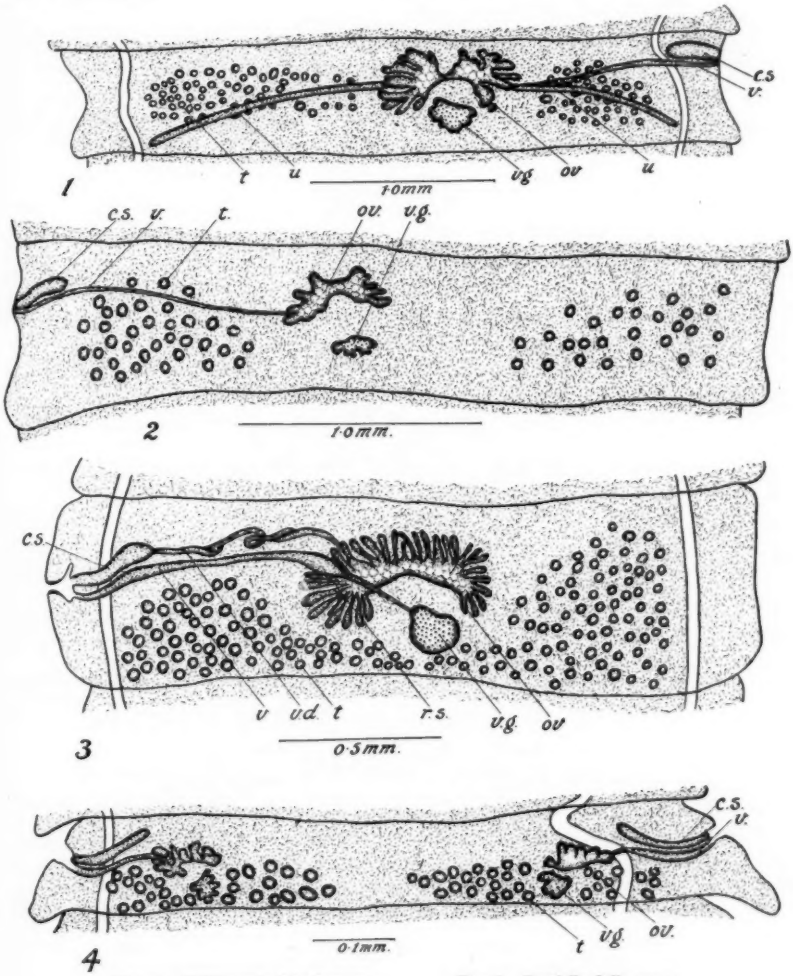
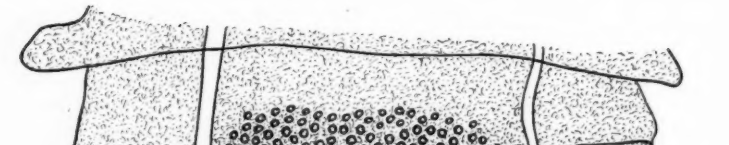


Fig. 1. *Killigrewia frivola* n.sp.
Fig. 2. *K. pamelae* n.sp.
Fig. 3. *Bertiella fallax* n.sp.
Fig. 4. *Cotugnia fleari* n.sp.



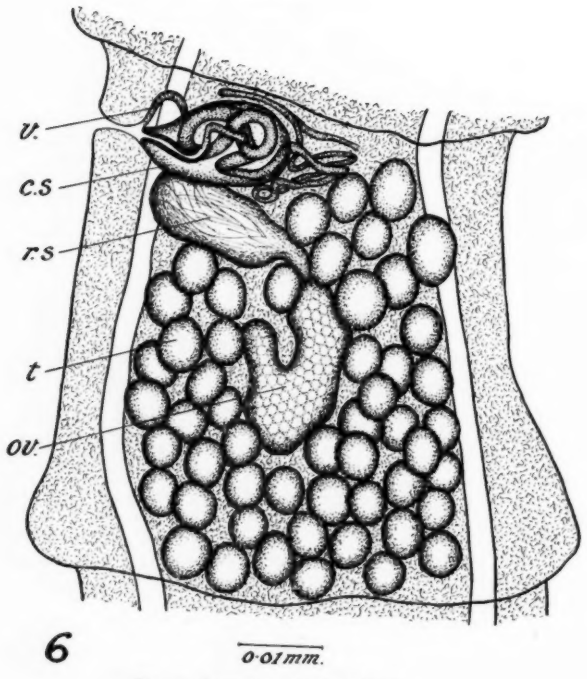


Fig 5. *Inermicapsifer otidis* n.sp.
Fig. 6. *Multitesticulata aegyptica* n.sp.

REFERENCE LETTERS.

c.s., Cirrus sac. *ov.*, Ovary. *r.s.*, Receptaculum seminis. *t.*, Testes. *v.*, Vagina. *v.d.*, Vas deferens. *v.g.*, Vitelline gland. *u.*, Uterus.

827 *a*. **I. nana** Fuhrmann, 1925.

Host. *Otis houbara*.

Locality. Egypt.

Scolex 0·08 dia.; rostellum 0·05 dia.; rostellar hooks 0·009 long. Cirrus sac 0·35 long, directed anteriorly, nearly reaching anterior margin of proglottis and aporal excretory vessel. Testes 12. Vagina coiled. At the present time six species of *Idiogenes* have been recorded: *I. flagellum* (Goeze, 1782), *I. grandiporus* Cholodkovsky, 1905, *I. horridus* Fuhrmann, 1909, *I. horridus africanus* Hungerbühler, 1910, *I. otidis* Krabbe, 1869, and *I. otidis nana* Fuhrmann, 1925. The table (p. 325) shows the differences between them. *I. otidis nana* differs from *I. otidis* in the number of testes (Fuhrmann, 1925, fig. 4, p. 148, testes 9–11; Zschokke, 1888, p. 121, testes 10–15 for *I. otidis*), the larger size of the cirrus sac and, if Krabbe (1869, p. 304) be correct in stating the rostellar hooks of *I. otidis* are 0·024–0·026 long, in the smaller size of the hooks; *I. nana* is therefore a distinct species.

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CARCINOMA IN APPOSITION TO *CYSTICERCUS FASCIOLARIS* IN A MOUSE INJECTED WITH CANCER CELLS.

By H. P. BAYON.

(*From the Molteno Institute for Research in Parasitology, Cambridge.*)

(With 5 Text-figures.)

The development of tumours in tissues invaded by certain helminthic parasites has been repeatedly observed, both clinically and experimentally; and though the exact mode in which various parasites give rise to malignant proliferations is still obscure, there is a tendency to conclude that they act in a purely mechanical and irritative fashion.

The strobiloid larva of *Taenia crassicollis* has been known to induce the formation of sarcoma in the liver of rats, both in nature and experimentally, but not in mice. In support of this view one need only quote Leitch (1923):

> It is presumed that the toxic products of the cysticercus furnish the necessary constant tissue irritant; but however that may be, a susceptible tissue is required. The same parasite is commonly found in the liver of mice, but sarcoma of the liver has never been reported in the mouse, save in one doubtful case, and neither in that, nor in the epithelial tumours of the liver, some thirty in number, could any parasite be discovered.

The abdominal tumour (in a mouse) the development of which is here described, was not a spontaneous neoplasm; and, since the parasitising of mice with *Taenia crassicollis* is an uncertain experiment, this observation is published now, independently of several analogous results with the same and other neoplasms. It may suggest an explanation of the factors influencing the localisation of growths connected with helminths; for the transplantation of the carcinoma was obtained with a dilute, filtered cell-suspension, which gave no results after injection into the peritoneal cavity of numerous other mice.

That, as a rule, tumour fragments do not take in rats and mice when implanted or injected intraperitoneally has been repeatedly noted (Jones and Rous, 1914).

Preparation of the Cell-suspension.

The filtered diluted cellular suspension injected in these experiments was obtained in the following fashion:

About half a gramme of fresh Bashford's No. 63 mouse-adeno-carcinoma was thoroughly ground up with fine sand in a mortar and 10 c.c. of Ringer's

solution added; the emulsion was strained through glass-wool, afterwards through a fused-glass filter, only a slight vacuum (50–70 mm. Hg) being employed. A drop was examined microscopically and, as was to be expected, revealed very numerous intact cells, either singly or in groups of twos and threes or more, the size and number of the minute tissue tatters depending above all upon the care and thoroughness of the preliminary grinding and being influenced only to a minor extent, within certain limits, by the closeness of the filter pores. By addition of a corresponding amount of Ringer's solution, the emulsion was adjusted so as not to contain more than 15,000 cells or cell-groups per c.c. This procedure could give approximate results, but in the experiments which are now being continued on the same lines, more exact measurements are being attempted.

Half a cubic centimetre was then injected into batches of mice, either intraperitoneally or into the loose subcutaneous tissue, with a graduated tuberculine syringe and thin hypodermic needle. No clogging resulted, proving that the suspension contained no obvious cell aggregates.

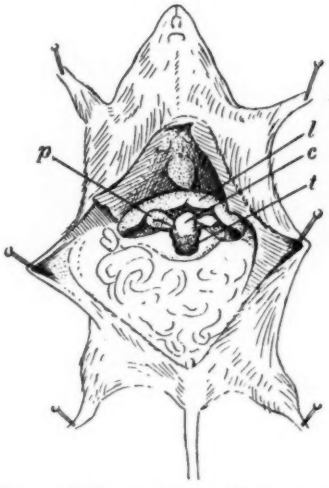


Fig. 1. Sketch of mouse at autopsy showing relative positions of cyst (*c*) above and adherent to tumour (*t*); the parasite (*p*) protruding through incisions; the whole overlapped by liver (*l*). (*H. C. Gillings del.*)

Experimental Notes.

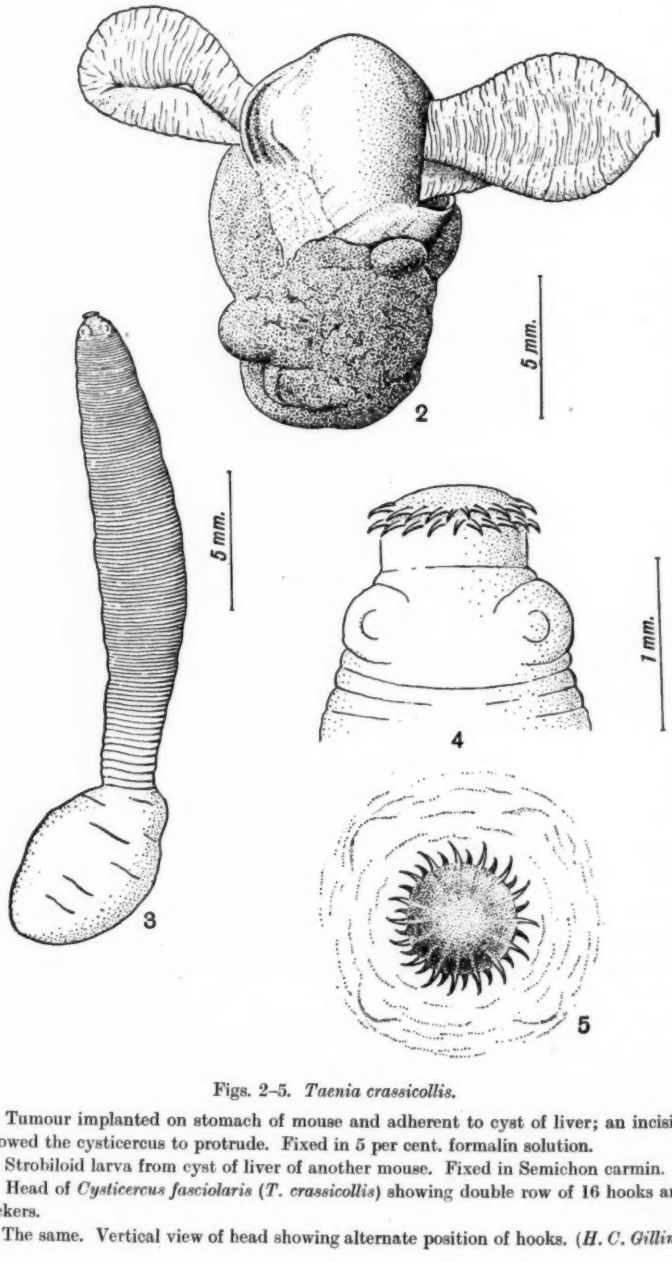
Fawn-coloured mouse No. 72.

October 15th, 1926. Injected intraperitoneally, right, 0·5 c.c. filtered cell suspension of carcinoma from operation on mouse 27.

November 24th, 1926. Injected same quantity of a similar suspension, left, from mice 27 and 31.

January 17th, 1927. Inoculated sacral region small piece carcinoma tissue from operation on mouse 70.

Found dead on January 27th, 1927. Autopsy same day.



Figs. 2–5. *Taenia crassicollis.*

Fig. 2. Tumour implanted on stomach of mouse and adherent to cyst of liver; an incision has allowed the cysticercus to protrude. Fixed in 5 per cent. formalin solution.

Fig. 3. Strobiloid larva from cyst of liver of another mouse. Fixed in Semichon carmin.

Fig. 4. Head of *Cysticercus fasciolaris* (*T. crassicollis*) showing double row of 16 hooks and four suckers.

Fig. 5. The same. Vertical view of head showing alternate position of hooks. (*H. C. Gillings del.*)

No scars, sores or superficial swellings detectable, except small lump over sacral region, which on incision is found to contain dry, cheesy cellular matter; microscopically necrosed cells.

Abdominal cavity smooth and glistening; intestines, liver, spleen and kidneys not abnormally congested. Liver contains a *C. fasciolaris* cyst and in its immediate neighbourhood and slightly adherent, is a sessile, pea-sized tumour originating apparently from the walls of the pyloric end of the stomach (see Figs. 1 and 2). In the right groin a subcutaneous small soft pink nodule, red on section; also a small hard gland. Lungs pink, free from metastasis.

Microscopically the tumour was found to consist of closely knitted adenomatous cells, corresponding in size, nucleus, arrangement, staining and general appearance to those well-known to distinguish Bashford's No. 63 mouse-adeno-carcinoma.

Much of the tissue stained badly or not at all, as it was undergoing various stages of necrosis and degeneration. The peritoneum is not a good site for the implantation of this class of tumours and in this case the graft only succeeded because specially favourable circumstances had been prepared in the abdominal cavity by the presence of the parasite.

Discussion.

The essential result of this experimental observation is, that, notwithstanding thirty-five other mice were injected in the same fashion on different occasions and watched over prolonged periods, only this mouse, which harboured a parasite known to give rise to malignant proliferations in the rat, developed a successful graft in the peritoneum in close proximity to the cyst.

Admitting that a small group of cells may have slipped through the fine hypodermic needle, the implantation in the immediate neighbourhood of the cysticercus is remarkable, for the other mice either resorbed the cells or on autopsy showed more or less extensive, scattered deposits of cellular remnants in various stages of necrotic disintegration, but no intact cells or organised tissue formation, other than an inflammatory reaction.

Similar partially necrosed grafts were produced in the peritoneum of mice by Jones and Rous (1914) by previously irritating and injuring the peritoneal cavity by finely ground diatomaceous earth, small glass rods and other mechanical means.

It may be added that in mice of another series, which were injected hypodermically in the mammary region with 0·2 c.c. of a similar cell suspension of Bashford's No. 63 mouse-adeno-carcinoma, two out of eleven animals developed tumours in the needle track, which proved to be of the same type as the one injected. The tumours in these two mice gradually increased in size and proved transplantable.

Preliminary experiments of this nature can only obtain permanent value through repeated confirmation. They cannot be expected to prove, or disprove, any views on the *causa causans* of cancer in man; though, when suitably correlated with other analogous experimental findings, they may support the theory that a susceptible tissue is required where tumours develop through the action of helminths and that the cause resides in the cells of the host and not in the parasite.

SUMMARY.

A mouse, which had been intraperitoneally injected with a filtered, diluted, cellular suspension of mouse-adeno-carcinoma, three months afterwards, was found to have developed a tumour of the same type on the pyloric end of the stomach surface, adherent to a cyst of the liver, containing the strobiloid larval stage of *Taenia crassicollis*.

Thirty-five other mice, similarly injected, but which were not infected with this cestode, did not develop growths in the peritoneum.

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REMARQUE À PROPOS DU MÉMOIRE DE A. W. SELLARDS ET W. A. BAETJER (1927), THE OCCURRENCE OF ATYPICAL AMOEBIASIS.

(*Parasitology*, XIX. 48–53, Plate I.)

PAR PROF. A. ALEXEIEFF.

DANS le pus des ulcérations du gros intestin on trouve toujours en nombre plus ou moins considérable des macrophages d'un type spécial. On rencontre ces macrophages dans la dysenterie bacillaire, dans la dysenterie amibienne, dans le colitis ulcerosa (cette dernière a, pour moi, une origine non infectieuse).

Les macrophages dont il s'agit ici proviennent non pas des monocytes comme c'est le cas général, mais des Plasmazellen d'Unna. J'ai décrit ailleurs[1] ces éléments; ici je vais rappeler très brièvement leurs traits essentiels.

Nous partons d'un élément lymphoïde ressemblant au petit lymphocyte, ou dans d'autres cas au lymphoblaste (*v.* mon travail cité ci-dessus, Pl. I, fig. 25). Le noyau de cet élément lymphoïde subit une pycnose périphérique; le plasma s'accroît et devient très basophile; des corpuscules de Russell y apparaissent. Nous avons les plasmocytes avec leur noyau excentrique et la structure caractéristique. Le noyau se fragmente et après ce caryorrhexis on trouve dans le cytoplasme autant de sphérules de chromatine qu'il y avait de blocs périphériques. Un certain nombre parmi ces plasmocytes fonctionnent comme macrophages: en effet ils ingèrent des leucocytes et plus rarement des hématies. Cette ingestion se fait tantôt suivant le mode unipolaire, tantôt bipolaire, et enfin parfois multipolaire.

Lorsque ces plasmophages (le terme que j'ai proposé pour distinguer ces macrophages d'avec les monophages—de beaucoup les plus communs) renferment quelques hématies ingérées la confusion avec l'*Entamoeba dysenteriae* est à craindre. Cette confusion a été maintes fois commise; ainsi Schaudinn a décrit la formation de 8 blocs de chromatine à la périphérie du noyau de l'amibe dysentérique; or dans ses figures on reconnaît les macrophages. Hartmann a pris ces derniers pour l'amibe dysentérique en dégénérescence. Brumpt donne de bonnes figures des macrophages et il les prend pour "l'*Endolimax phagocytoides*" Gauducheau.

Une erreur du même genre vient d'être faite par Sellards et Baetjer. La position du noyau, la structure "atypique" de celui-ci—tout montre que dans

[1] Alexeieff (1926). Recherches sur la physiologie des globules blancs. Cytodiagnostic et son application en clinique, *Centralbl. f. Bakt.* **101**, Heft 4/5.

les formations figurées par ces auteurs il faut voir des plasmophages. Sellards et Baetjer avaient un cas de colite ulcéreuse et non de dysenterie amibienne.

Cette erreur regrettable ne devrait plus avoir lieu d'autant plus que précisément dans la littérature anglaise il y a un travail où sont décrits ces macrophages. Je fais allusion au mémoire de J. Gordon Thomson (1926), Pseudoparasites in the faeces of man, *Proc. Roy. Soc. Med.* **19**.

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GRAYALLIA QUADRISPINA, A NEW GENUS AND SPECIES OF MONOCYSTID GREGARINE PARASITE IN AN OLIGOCHAETE, *PHERETIMA HETEROCHAETA* (MICH.).

By S. B. SETNA, M.Sc.

(*Zoological Laboratory, Cambridge.*)

(With 3 Text-figures.)

The monocystid here described was found in the coelom of *Pheretima heterochaeta* (Mich.).

The following account is based wholly on material fixed in vapour of osmic acid and methyl alcohol—other fixatives like Bouin's and Schaudinn's gave bad results.

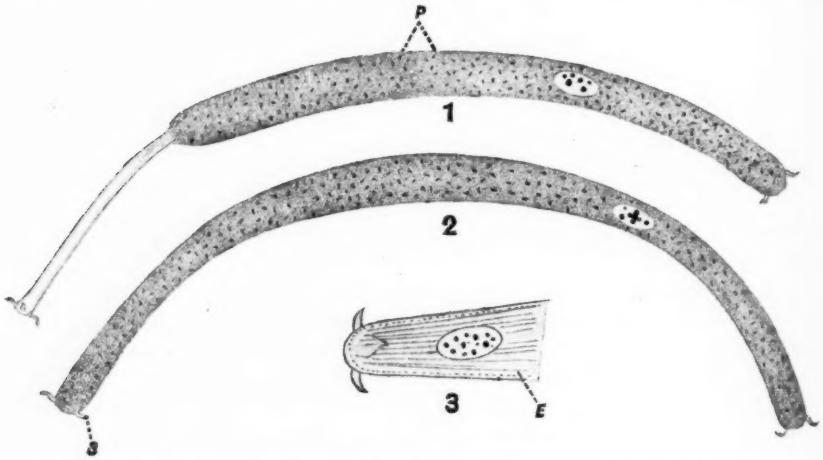
The gregarine was encountered on several occasions lying free in the coelom of the host. A careful examination was made and sections of the alimentary canal of heavily parasitised hosts were cut, but so far, I have been unable to identify any attached form. The cysts and sporocysts of this, as well as those of another gregarine, occur in the coelom, and, since the cysts and sporocysts of the various Monocystids resemble one another very closely, it was found impossible to separate the developmental stages of this particular species.

The trophozoites are long and slender; one very large individual measured 1270μ by 36μ. The most remarkable feature is the presence of 4 spines at either end, a character not known to occur in any other Monocystid. The spine-like prolongations arise from the surface of the body at a little distance from the extremities; they are broader at the base than at the apex and are subequal, measuring ca. 12μ in length. The spines are capable of movement, being either swung actively from side to side or held straight against the body in vigorous specimens; they occur in every individual and their function cannot as yet be explained.

The endoplasm of this parasite is very granular and mobile. Owing to the very active movements of the endoplasm, the parasite assumes a variety of appearances. When extended, the breadth is comparatively uniform (Fig. 2), but when the parasite contracts a characteristic shape is assumed as shown in Fig. 1. The epicytal striations are well developed over the general body-surface; they are faintly visible and lie close together on the spines. The sarcocyte layer is feebly developed and the layer of myocyte is also thin; these

layers are both continued into the prolongations. The endoplasm is not continued into the spines, so that in their structure, and to some extent in form, they recall the spines in the genus *Dirhynchocystis* described by Cognetti de Martiis (1920) and Bhatia and Chatterji (1925).

In the endoplasm are lodged paraglycogen grains which with iron haematoxylin stain darker than the rest of the body. Besides these are present many small oval and sometimes rod-shaped bodies—which may be parasites—distributed evenly throughout the cytoplasm, being far more abundant in larger individuals. The occurrence of parasites in gregarines has been reported by Porter in *Amaroucium*, by Caullery and Mesnil in certain gregarines of the



Grayallia quadrispina. Free hand sketches from permanent preparations fixed in vapour of osmic acid and methyl alcohol and stained with iron haematoxylin.

Fig. 1. Adult trophozoite fixed while contracting.

Fig. 2. Adult uncontracted trophozoite fixed when motionless, breadth uniform.

Fig. 3. End of adult trophozoite highly magnified, showing epicytal striations. The karyosomes, of which there are several, are scattered.

P = Parasitic inclusions? S = Spines. E = Epicytal striations.

Annelids, Brasil and Fantham in the Selenidiidae, etc., so that the phenomenon is fairly well known.

The nucleus of the trophozoite is elongate ellipsoid with a variable number of karyosomes which in some nuclei are found assembled in the centre. In most nuclei the karyosomes are distinct in outline and discrete. The nucleus in living trophozoites usually lies towards the extremities.

The parasite belongs to the family Monocystidae and is, I believe, now recorded for the first time. It exhibits a very striking feature in the possession of spines and requires a new genus for its reception. I therefore propose to name the genus *Grayallia* and the species *quadrispina*.

Diagnosis. Nematocystis-like form, attaining a length of about 1270μ with 4 spine-like processes arising from the surface of the body at either end and radiating backwards. Nucleus large and oval, containing a variable number of karyosomes. Trophozoites chalky white in colour and highly deformable. Cysts and sporocysts have not been identified. The dimensions of the trophozoite and its various parts *in microns*.

	Length of trophozoite	Breadth of trophozoite	Nuclear diameter	No. of karyosomes and size of individual karyosomes
1	720	50	34 by 16	
2	800	40	24 by 14	5 karyosomes each 3
3	850	50	28 by 15	15 karyosomes all of different sizes, some mere dots
4	900	35	27 by 15	2 karyosomes 8 each
5	1000	40	32 by 18	2 karyosomes (1) 8×8, (2) 6×6
6	1050	40	40 by 12	8 karyosomes $2\frac{1}{2}$ each
7	1270	35	Nucleus not distinct	
8	900	40	34 by 18	5 karyosomes each $2\frac{1}{2}$

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A REFERENCE LIST OF THE TREMATODE PARASITES OF MAN AND THE PRIMATES.

By WILLIAM NICOLL, M.A., D.Sc., M.D.

Virol Pathological Research Laboratories, London.

The Trematode parasites of man have been so frequently listed and, for the most part, so fully described in various text-books and treatises on parasitology, pathology and tropical medicine that it might seem superfluous to enumerate them again. My object in doing so is to provide a basis for comparison with the parasites of the Primates, our knowledge of which is still extremely fragmentary, and with the parasites of Mammals in general. It is hoped that the works which are cited will prove to be the most useful or the most authoritative original references available.

During the latter half of last century the best-known standard work on the parasitic worms of man was that of Rudolf Leuckart but within recent years its place has been taken by the more modern text-book of Max Braun (Braun and Seifert, 1925). In France for many years the standard text-book was Blanchard's *Traité de Zoologie médicale* (1888), but this has been superseded by several more up-to-date works, *e.g.* those of Brumpt, Neveu-Lemaire, etc. In English the most recent works are Fantham, Stephens, and Theobald's *Animal Parasites of Man* (1916) and Chandler's *Animal Parasites and Human Disease* (1918). In addition the animal parasites of man receive adequate treatment in most text-books of tropical medicine such as Mense's *Handbuch der Tropenkrankheiten*, Castellani and Chalmers' *Manual of Tropical Medicine*, Manson's *Tropical Diseases*, Byam and Archibald's *The Practice of Medicine in the Tropics* and in various text-books of general medicine and pathology. The most complete bibliography of the subject is to be found in Stiles and Hassall's *Index Catalogue of Medical and Veterinary Zoology*.

To the general medical practitioner in Europe the Trematode parasites of man are unlikely to be of more than academic interest, but to the practitioner in tropical and certain sub-tropical countries they may become a matter of considerable practical importance. The endemic prevalence of bilharziasis (schistosomiasis) in Northern Africa and Eastern Asia and of clonorchiasis in the latter region are matters of grave concern to sanitarians, and the sporadic invasion of certain parts of Southern Europe, America and South Africa by bilharziasis has become a subject of general interest to epidemiologists.

In spite of numerous investigations it is still to be admitted that our knowledge of several parasites of man remains incomplete and defective in regard not only to structure but more particularly to life history, bionomics and pathogenicity. Inaccuracies in the description of structural details have given rise to confusion of various species with the result that in some cases two or more species have become merged under the same name or, more frequently perhaps, the same species has been described under several different names by different observers at various times. It is for these reasons that identification of a particular species not infrequently presents undue difficulty to the non-specialist who lacks access to an extensive helminthological library, and it may be on this account that many medical men in the tropics abandon any attempt at precise identification.

The chief difficulties in field helminthology are, I believe, failure to realize on the one hand that many of the parasites which infest man may also be found in other Mammals, and, on the other, that various Mammals are frequently infected with parasitic species closely resembling, but actually different from, those occurring in man. These are reasons which may, in some cases, be held to account for the superfluity and confusing redundancy of synonyms in the nomenclature of certain parasites of man.

On purely zoological grounds one might have expected a fairly close correspondence between the parasites of man and those of the other Primates, but this is not particularly manifest in the case of Trematodes, so far as our present knowledge goes. Thus the only records of Trematodes which occur in man being also found in monkeys are the somewhat doubtful cases of *Schistosoma haematobium* in the sooty monkey (*Cercocebus fuliginosus*), *Watsonius watsoni* in *Cercopithecus callitrichus*, and the possibly identical *Watsonius macaci* in *Macaca cynomolga*.

It is rather curious to find occurring in monkeys as many as three different species of the family *Lecithodendriidae*, a group which is so typically associated with bats. Noteworthy also is the record of the Amphistome species, *Chiorchis noci*, in *Macaca cynomolga*.

The species which may be regarded as fairly common or characteristic parasites of man are *Clonorchis sinensis*, *Opisthorchis felineus*, *Fasciolopsis buski*, *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Paragonimus westermani*, *Schistosoma haematobium*, *S. japonicum* and *S. mansoni*, but all these species except the last-mentioned have been recorded from other animals in addition, viz.:

	Hosts
Clonorchis sinensis	Cat, dog, pig, rat, mouse, marten, badger, and other mammals
Opisthorchis felineus	Cat, dog, pig, fox and glutton
Fasciolopsis buski	Pig
Heterophyes heterophyes	Cat, dog, fox, and wolf
Metagonimus yokogawai	Cat, dog and pig
Paragonimus westermani	Cat, dog, tiger and mountain lion
Schistosoma haematobium	Sooty monkey
Schistosoma japonicum	Cat, dog, horse, rats, mice and other mammals

The other species which have, from time to time, been recorded as occurring in man are probably only to be regarded as incidental though it is not known in every case what are the natural hosts of each particular species. In the case of the following species, however, the probable natural hosts are:

	Hosts
Opisthorchis viverrini	Civet cat
Opisthorchis tenuicollis	Seals
Amphimerus noverca	American fox
Fasciola hepatica	Cattle, sheep and other Herbivora
Fasciola gigantica	Giraffe
Dicrocoelium lanceatum	Cattle, sheep and pigs
Gastrodiscoides hominis	Monkeys, pigs and deer
Watsonius watsoni	Monkeys
Stamnosoma armatum	Various mammals and birds
Stamnosoma formosanum	Dog, cat, etc.
Monorchotrema taihokui	Dog, cat, etc.

It would, thus, appear that there are few, if in fact any, species of Trematodes, which can be regarded as exclusively specific parasites of man, and it is indeed open to doubt if any are absolutely specific for the Primates even in nature. It would certainly appear that the dog and possibly also the cat are at least susceptible to infection with most of the species which commonly infest man. A comparison of the list of the parasites of man with those of the dog and the cat shows that the only species which have not been recorded in one or other of the latter are *Fasciola hepatica*, *Opisthorchis viverrini*, *Schistosoma haematobium*, *S. mansoni* and the Amphistomes.

Since the introduction of the system of binary zoological nomenclature the first report of the occurrence of a Trematode parasite in man appears to be the record of *Fasciola hepatica* (*F. humana*) by Gmelin in 1790 and the first record from an ape that of *Fasciola maimonis* by de Blainville in 1828. *Dicrocoelium lanceatum* appears to have been first reported in man in 1803, but nearly fifty years elapsed before a third species, *Schistosoma haematobium*, was made known by Bilharz in 1852, and, in the following year, *Heterophyes heterophyes* was discovered by von Siebold. *Fasciolopsis buski* was described by Lankester in 1857 but there was an interval of eighteen years before *Clonorchis sinensis* became known through Cobbold. A year later came the discovery of *Gastrodiscoides hominis* by Lewis and McConnell and two years later (1878) *Paragonimus westermani* was first made known to the Western world. The most recent additions to the list of the Trematode parasites of man are two small intestinal flukes *Stamnosoma formosanum* and *Monorchotrema taihokui* discovered by Nishigori in Formosa.

None of the earlier helminthologists appear to have had much experience of the Trematode parasites of the Primates other than man, and our knowledge of these may really be dated from the time of Diesing (1839–1855) who, in 1839, described *Amphistoma emarginatum* from a Brazilian monkey.

So far as medical helminthology is concerned real interest in the Trematoda as causal factors in disease may be taken to date from the time of Bilharz

and von Siebold (1852). In this country the first outstanding medical helminthologist was Cobbold (1855–1879) whose name is associated chiefly with *Heterophyes* and *Clonorchis*. His work, however, was overshadowed by that of Leuckart (1863–1889), probably the best known of all helminthologists who have devoted themselves to the special problems affecting man.

Equally well known in France was the versatile Raphael Blanchard (1888–1896) whose name, in Trematode literature, is identified most closely with the genus *Opisthorchis*. The fourth of the great medical helminthologists of the latter half of the nineteenth century was Max Braun (1893–1902), who was responsible for the generic name *Paragonimus*.

The beginning of the present century found Looss (1894–1913) established as the leading authority on Trematodes and he has probably influenced medical opinion in regard to these parasites even more than did Leuckart or Cobbold.

During the past twenty-five years our knowledge of the Trematode parasites of man and the Primates has been extended chiefly through the researches of Stiles (1894–1910), Katsurada (1900–13), Odhner (1902–14), Leiper (1911–18), Kobayashi (1911–20), Faust (1920–6) and Nishigori (1924–6). Stiles' work has been devoted principally to the taxonomy of the Amphistomes and to the effort to secure uniformity and, above all, stability in nomenclature. To Katsurada lies the credit of much valuable pioneer work on the Japanese Schistosome. Odhner has dealt with *Fasciolopsis*, *Paragonimus* and the Echinostomes of man. Kobayashi and Nishigori have made interesting additions to the Trematode fauna of Japan. Leiper's chief contribution has been to our knowledge of *Schistosoma haematobium* while Faust has been responsible for the most comprehensive accounts of the Japanese Schistosome and of *Clonorchis sinensis*.

During the past year two publications of the highest importance in systematic helminthology have appeared, namely, Stiles and Hassall's *Key Catalogue of the Worms reported for Man* and Poche's *System der Platodaria*. Stiles and Hassall confine themselves, so far as possible, to the exposition and elucidation of already existing schemes of classification and nomenclature, but Poche has essayed to rehabilitate almost the entire fabric of Trematode taxonomy. This affects the parasites of the Primates in certain details, to which I shall briefly refer.

Until comparatively recently the general broad division of the digenetic Trematodes into five or six chief groups, viz. *Distomata*, *Monostomata*, *Amphistomata*, *Gasterostomata*, *Holostomata* and *Schistosomata*, has been accepted by most writers on the subject. These groups, as their names indicate, were based on certain outstanding structural features, *e.g.* the presence of only one sucker in the *Monostomata*, the sexual dimorphism in the *Schistosomata* and so forth. The taxonomic status of these units has provided much occasion for speculation, discussion and even controversy, but the question of their convenience has rarely been seriously raised. The systematic evaluation of

the individual groups, however, has varied from time to time. By some authorities they have been assigned the rank only of families, *e.g. Distomidae, Monostomidae*; by others they have been considered as super-families, *e.g. Paramphistomoidea*, but Poche has raised them a step further and designated them super-super-families and even, as in the case of the *Gasterostomata*, sub-orders.

It is not my intention to enter here into criticism of these important considerations. They are mentioned merely in order to account for the introduction of certain taxonomic terms which have not yet found their way into helminthological publications in general.

Arising from them, however, are one or two points of immediate interest, more especially in regard to nomenclature. Both Stiles and Poche are well known in the zoological world as two of the leading spirits in the present-day urge for nomenclatural exactitude in the biological sciences and more especially in zoology. So far as helminthology is concerned Stiles has come more prominently into notice. His efforts to secure stability have been rightly appraised but his excursions into the details of terminology and taxonomy have not met with unreserved approval.

It is unnecessary here to recapitulate all the grounds on which the system of taxonomy, formulated by Stiles, has been arraigned. It is sufficient to indicate that it has been condemned as essentially superficial, artificial and unnatural, and that it has found but little favour outside America.

Of the various critics of Stiles' system the most formidable were Looss and Odhner, and their views have been upheld and embellished by Poche in what is probably the most intriguing work on the Trematoda since the appearance of Looss's momentous *Weitere Beiträge* in 1899.

Poche's system does not materially affect the detailed classification of the Trematode parasites of the Primates, but he has introduced certain modifications and variants of previously recognised groups. All the Trematode parasites of the Primates are assigned to the sub-order *Prosostomata* and the tribe *Fascioloidae*. The latter comprises ten super-families of which only three are represented amongst the Primates, namely the *Fasciolida*, the *Schistosomatida* and the *Paramphistomida*. There are three species of Schistosome and five of Paramphistome while all the remaining species belong to the group *Fasciolida*.

To the medical helminthologist who is not particularly versed in the conventions of zoological nomenclature some of Poche's emendations may appear pedantic and even inconsistent. Thus he alters the family name *Echinostomidae* Looss to *Echinostomatidae* nom. nov. in accordance with the principle that family names shall be formed by affixing the termination *-idae* to the root of the name of the type genus. In this particular case the root is not *stom* but *stomat*, hence *Echinostomatidae*. But elsewhere we find Poche proposing the name *Paramphistomida* in preference to *Paramphistomatida*. He explains this apparent inconsistency on the score that in the first instance

the type genus is *Echinostoma* and in the second *Paramphistomum*, the former having a Greek termination and the latter a Latinized one.

This appears plausible enough, if somewhat pedantic, but from a practical point of view it is not only ridiculous and undesirable, but, in fact, quite wrong. It is based on the assumption that all generic names are of Latin or Greek origin or at least have a Latin or Greek form. Now the word *stoma* is obviously Greek, but I am unaware that *stomum* is a word in the Latin language or for that matter in any language. It is therefore idle to speak of the Latin "root" or "stem" of this word in forming a family name derived from it. It has no Latin stem any more than it has an English stem. The only stem which it might be considered to have is that of the Greek *stoma* from which it is obviously derived. From this it would follow that family names, whether derived from generic names ending in *stoma* or in *stomum* should have the form *stomatidae*. The only alternative is to regard *stomum* as an artificially coined word having no root other than the complete word itself, in which case the family name would terminate in *stomumidae*.

It is obvious that Poche's emendations as applied to the Platyhelminthes will apply equally to the Nemathelminthes and we shall therefore be under the necessity of making the following alterations in certain family names, viz.:

Gnathostomatidae	nom. emend.	*pro*	*Gnathostomidae* R. Blanchard 1895
Gnathostomatinae	„	„	*Gnathostominae* Baylis and Lane 1920
Gongylonematinae	„	„	*Gongyloneminae* Hall 1916
Trichonematinae	„	„	*Trichoneminae* Railliet 1916
Ancylostomatidae	„	„	*Ancylostomidae* Looss 1905
Ancylostomatinae	„	„	*Ancylostominae* Looss 1905

In accordance with the same principles it will follow that the generic name *Cyathostomum* Molin 1861 is not invalidated by *Cyathostoma* E. Blanchard 1849, and that therefore the generic name *Trichonema* Cobbold 1874 must again lapse into synonymy. It also follows that the sub-family names *Trichoneminae* Railliet 1916 and *Trichonematinae mihi* (supra) must be replaced by *Cyathostominae* nom. nov.

In view of many differences of opinion which still exist in regard to a number of species it has been considered advisable to adopt as conservative an attitude as possible in the subjoined lists. The status of certain genera such as *Artyfechinostomum*, *Amphimerus* and *Brodenia* is very doubtful. *Artyfechinostomum* is possibly identical with *Euparyphium*, and *Amphimerus* is certainly only a variant of *Opisthorchis*, the difference being of little more than specific value. *Brodenia* appears to bear some resemblance to *Dicrocoelium*. Certain species included in the list are of doubtful validity, while in the case of others the synonymy is extensive and complicated. *Fasciolopsis buski*, for instance, has been described under at least five different names, but current opinion is that there is only one species. There has been even more confusion in regard to *Metagonimus yokogawai*. Within a year of its discovery this parasite had received at least three generic names and two

specific names, and one finds it referred to under various combinations of these generic and specific names. The fact that the original records of the species were published in somewhat inaccessible Japanese journals contributed materially to the confusion. The consensus of opinion, summed up in Stiles and Hassall's Index catalogue and more recently substantiated by Faust and Nishigori (1926), is to the effect that *Metagonimus yokogawai* Katsurada is the name by which the species should properly be designated.

The parasites of the monkeys and apes are not at all well known and the literature relating to them consists of little more than the original records, which in many cases are far from satisfactory. The chief difficulty, however, in dealing with the parasites of the Primates is to determine accurately in what precise species of host the parasites were found. The nomenclature of the hosts appears to be even more complicated than that of their parasites.

The references given in the subjoined lists are as far as possible to the primary records and to the most accurate or comprehensive of subsequent descriptions. References to the parasites of apes and monkeys are exceedingly few.

LIST OF THE TREMATODE PARASITES OF MAN AND THE PRIMATES, ARRANGED SYSTEMATICALLY.

I. Fasciolida Poche 1926.

I. Echinostomatidae (Looss 1902) Poche 1926

i. *Echinostoma* Rudolphi 1809

E. ilocanum (Garrison 1908) — Odhner 1911

ii. *Euparyphium* Dietz 1909

E. malayanum (Leiper 1911) — Odhner 1913, Leiper 1913

E. jassyense Leon and Ciurea 1922

iii. *Artyfechinostomum* Lane 1915

A. sufrartyfex Lane 1915

iv. *Echinochasmus* Dietz 1909

E. perfoliatus japonicus Tanabe 1919

II. Opisthorchidae (Braun 1901) Lühe 1901

i. *Opisthorchis* Blanchard 1895

O. felineus (Rivolta 1884) — Braun 1895, Barker 1911

O. viverrini (Poirier 1886) — Leiper 1915

O. (Amphimerus) noverca Braun 1902 — Braun 1903, Looss 1905

ii. *Clonorchis* Looss 1907

C. sinensis (Cobbold 1875) — Looss 1907, Kobayashi 1911, Faust and Khaw 1927

iii. *Pseudamphistomum* Lühe 1908

P. truncatum (Rud. 1819) — Braun 1893, Stiles and Hassall 1894

III. Fasciolidae Railliet 1895

i. *Fasciola* Linn. 1758

F. hepatica Linn. 1758 — Sommer 1880, Thomas 1883, Blanchard 1896

F. gigantica Cobbold 1855 — Faust 1920, Porter 1920, Jackson 1921

ii. *Fasciolopsis* Looss 1899
F. buski (Lankester 1857) — Odhner 1902, 1908, Ward 1909
[= F. spinifera Brown 1917 — Goddard 1919, Barlow 1925
IV. DICROCOELIIDAE Odhner 1910
i. *Dicrocoelium* Dujardin 1845
D. lanceatum Stiles and Hassall 1896 — Braun 1895, Anglas and Ribaucourt 1902
[= D. lanceolatum (Rud. 1803)
?= D. dendriticum (Rud. 1819) Odhner 1910]
D. macaci Kobayashi 1920
ii. *Eurytrema* Looss 1907
E. brumpti Railliet, Henry and Joyeux 1912
E. satoi Kobayashi 1920
iii. *Athesmia* Looss 1899
A. foxi Goldberger and Crane 1911
[= Hepatotrema cebi Stunkard 1923]
iv. *Brodenia* Gedoelst 1913
B. serrata Gedoelst 1913
B. maimonis (Blainv. 1828)
V. LECITHODENDRIIDAE Odhner 1910
i. *Phaneropsolus* Looss 1899
P. orbicularis (Diesing 1850) — Braun 1901
P. oviformis (Poirier 1886) — Looss 1899
P. longipenis Looss 1899 — Braun 1901
VI. HETEROPHYIDAE Odhner 1914
i. *Heterophyes* Cobbold 1866
H. heterophyes (v. Siebold 1852) — Looss 1894, 1902 *a*
[= H. nocens Onji and Nishio 1915] — Ransom 1920
H. katsuradai Ozaki and Asada 1925 — Ozaki and Asada 1926
ii. *Metagonimus* Katsurada 1913
[= *Loxotrema* Kobayashi 1912 = *Yokogawa* Leiper 1913]
M. yokogawai (Katsurada 1912) — Yokogawa 1913, Ransom 1920
[? = Loxotrema ovatum Kobayashi 1912]
iii. *Stamnosoma* Tanabe 1922
S. armatum Tanabe 1922
S. formosanum Nishigori 1924 — Nishigori 1924 *a*
[= Centrocestus cuspidatus *var.* caninus Leiper 1913]
iv. *Monorchotrema* Nishigori 1924
M. taihokui Nishigori 1924 — Nishigori 1924 *b*, Faust and Nishigori 1926
VII. TROGLOTREMATIDAE (Odhner 1914) Braun 1915
i. *Paragonimus* Braun 1899
P. westermani (Kerbert 1878) — Katsurada 1900, Kubo 1912, Ward and Hirsch 1915, Nakagawa 1918

II. SCHISTOSOMATIDA Poche 1926
= SCHISTOSOMATOIDEA Stiles and Hassall 1926.

I. SCHISTOSOMATIDAE (Looss 1899) Poche 1907
i. *Schistosoma* Weinland 1858
S. haematobium (Bilharz 1852) — Leiper 1915–1918, Looss 1895, Letulle 1905
S. japonicum Katsurada 1904 — Katsurada 1913, Faust and Meleney 1924
S. mansoni Sambon 1907 — Lutz 1917, Bahr and Fairley 1920

III. Paramphistomida Poche 1926.

I. Paramphistomidae Fischoeder 1901

i. *Paramphistomum* Fischoeder 1901

(P.) emarginatum Diesing 1839 — Diesing 1850, Fischoeder 1902

ii. *Watsonius* Stiles and Goldberger 1910

W. watsoni (Conyngham 1902) — Shipley 1905, Stiles and Goldberger 1910

W. macaci Kobayashi 1920
[? = W. watsoni Conyngham]

iii. *Chiorchis* Fischoeder 1901

C. noci Barrois 1908

II Gastrodiscidae Stiles and Goldberger 1910

i. *Gastrodiscoides* Leiper 1913

G. hominis (Lewis and McConnell 1876) — Stephens 1906, Leiper 1913, Lane 1924

IV. Immature and indeterminate forms.

Cercaria cercopitheci Cobbold 1861

Cercaria macaci Cobbold 1861

Agamodistomum ophthalmobium Diesing 1850 — Leuckart 1889

= ? Monostomum lentis Gescheidt 1833 — Stiles 1902, Ward 1903

= ? Dicrocoelium lanceatum Stiles and Hassall 1896

Distomum hematoma Semprun 1890 — Blanchard 1891, Braun 1891

Distomum intestinale Taylor 1862 — Carter 1862

LIST OF THE TREMATODE PARASITES OF THE PRIMATES, ARRANGED ACCORDING TO THEIR HOSTS.

1. *Homo sapiens* L.

Echinostoma ilocanum (Garr.)	Intestine
Euparyphium jassyense Leon and Ciurea	Intestine
Euparyphium malayanum (Leiper)	Intestine
Artyfechinostomum sufrartyfex Lane	Intestine
Echinochasmus perfoliatus japonicus Tanabe	Intestine
Opisthorchis felineus (Rud.)	Gall-bladder and bile ducts
Opisthorchis viverrini (Poir.)	Gall-bladder and bile ducts
Opisthorchis noverca Braun	Gall-bladder and bile ducts
Clonorchis sinensis (Cobbold)	Gall-bladder and bile ducts
? Pseudamphistomum truncatum (Rud.)	Gall-bladder and bile ducts
Fasciola hepatica Linn.	Liver and bile ducts
Fasciola gigantica Cobbold	Lungs
Fasciolopsis buski Lank.	Intestine
Dicrocoelium lanceatum Stiles and Hassall	Liver and bile ducts
Heterophyes heterophyes (v. Sieb.)	Intestine
Heterophyes katsuradai Ozaki and Asada	Intestine
Metagonimus yokogawai (Katsurada)	Intestine
Stamnosoma armatum Tanabe	Intestine
Stamnosoma formosanum Nishigori	Intestine
Monorchotrema taihokui Nishigori	Intestine
Paragonimus westermani (Kerbert)	Lungs
Schistosoma haematobium (Bilharz)	Portal and mesenteric veins
Schistosoma japonicum Katsurada	Portal and mesenteric veins
Schistosoma mansoni Sambon	Portal and mesenteric veins

Watsonius watsoni (Conyngham)	Intestine
Gastrodiscoides hominis (Lewis and McConnell)	Intestine and caecum
"Agamodistomum ophthalmobium" Dies.	Eye
"Distomum hematoma" Semprun	Blood
"Distomum intestinale" Taylor	Intestine
2. *Anthropopithecus troglodytes* (Blumb.)	
Eurytrema brumpti Raill. and Henry	Liver and pancreas
3. *Papio maimon* L.	
Brodenia maimonis (Blainv.)	Pancreas
? = Fasciola laciniata Duj.	
4. *Macaca cynomolga* L.	
Eurytrema satoi Kobay.	Pancreas
Watsonius macaci Kobay.	Caecum
Chiorchis noci Barrois	Caecum
5. *Macaca fuscata* Blg.	
Dicrocoelium macaci Kobay.	Liver
6. *Macaca radiata* Geoff.	
Cercaria macaci Cobb.	
7. *Cercopithecus callitrichus* Geoff.	
Watsonius watsoni Conyngh.	Intestine
8. *Cercocebus fuliginosus* Geoff.	
Schistosoma haematobium (Bilharz)	Portal veins
Cercaria cercopitheci Cobb.	
9. *Cercocebus* sp. [Belgian Congo]	
Brodenia serrata Gedoelst	Pancreas
10. *Nyctipithecus trivirgatus* Humboldt	
Phaneropsolus orbicularis (Dies.)	Intestine
Paramphistomum emarginatum (Dies.)	Intestine
11. *Nycticebus javanicus* Geoff.	
Phaneropsolus oviformis (Dies.)	Intestine
12. *Cebus apella* L.	
Athesmia foxi Goldb. and Crane	Liver
13. *Cebus capucinus* Erxl.	
Athesmia foxi Goldb. and Crane	Liver
14. "*Ape*" [Egypt]	
Phaneropsolus longipenis Looss	Intestine

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A PARASITIC FIBROMA ON EQUINES IN SAMOA.

By PATRICK A. BUXTON.

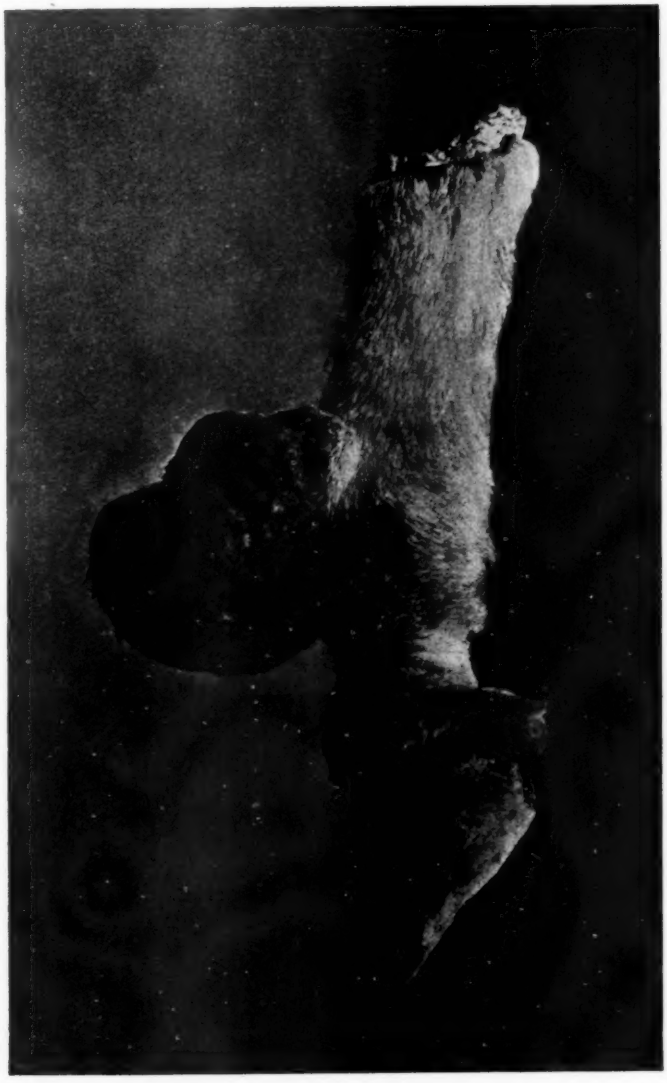
London School of Hygiene and Tropical Medicine.

(With Plate XXIII.)

In the Samoan Islands, in the South Pacific, one frequently sees horses and donkeys disfigured by large tumours. These occur on the pasterns and fetlocks, and occasionally between the eye and the nostril, but never on other parts of the body. They are often multiple, and they cause much inconvenience because of their size; many of them are larger than the animal's hoof (Plate XXIII).

I have examined a number of these tumours, on living and dead animals, and found that they do not invade bones or joints; they are uniformly hard fibromata, and they bleed very little if they are cut. When they are large they frequently break down, and part sloughs away, leaving a chronic ulcer. No organisms were found in smears from the cut surface, nor in dissections of the tumours; no granules or other indications of Mycetoma were discovered; the great majority of sections showed nothing but a very dense mass of avascular fibrous tissue, but pieces of a Nematode were occasionally seen. Prof. R. T. Leiper, F.R.S., to whom I showed the sections, was unable to identify the worm; it does not possess the cuticular rugae, characteristic of *Onchocerca*, and it is possible that it is a saprophytic organism which has invaded the pre-existing fibroma. On the other hand it seems probable that the tumour is caused by some parasite, because its occurrence seemed to be localised to certain parts of the island, and certain parts of the body of the host.

Whatever the cause, this disease cannot be native to Samoa, because horses were first imported about 1840; as the only native mammals are three species of bat, and one rat, it is most unlikely that it has spread from them to the equines. But though the condition is so conspicuous I have failed to discover that it prevails elsewhere in the world, though I have made enquiries of a number of British and American veterinary authorities. I understand that it is not found in Fiji, and I saw no cases in the New Hebrides.



PARASITIC FIBROMA ON FORE-LEG OF DONKEY.

NOTES ON THE GENUS *LEUCOCHLORIDIUM* CARUS (TREMATODA).

By ALLEN McINTOSH.

Department of Zoology, University of Minnesota.

(With 5 Text-figures.)

Introduction.

During the period from 1924 to 1926, in which occasional examinations of a few of our native birds for trematode parasites were made, five species of birds were found to be infested with flukes belonging to the genus *Leucochloridium* Carus. Since no adult forms of this genus have been reported heretofore from North America, it was thought advisable to make a thorough study of these parasites. This was done both from whole mounts, and, in all but one case, from cross sections as well. The material was found to represent five species new to science. These species are described and figured below, and a key for separating the known species of *Leucochloridium* is given.

The genus *Leucochloridium* is known primarily in the immature stages, which occur as highly coloured banded sporocysts in the tentacles of snails (*Succinea* and *Planorbis* in America according to Magath). The adults are parasitic in the intestine of birds, and the genus is evidently represented in this country by a number of species. However, very little work has been done in America on avian trematodes, and the few records available mostly pertain to fish-eating birds. The fact that many unknown host relationships remain to be recorded and new species to be described, should be an incentive for further investigation in this field.

Acknowledgements are due to Dr William A. Riley, Head of the Department of Zoology and Professor of Parasitology, for the suggestion of the work on which this paper is based and for advice and help while the work was in progress.

History of Genus.

The genus *Leucochloridium* was created by Carus in 1835 to contain a larval trematode from a snail belonging to the genus *Succinea*. The first record of an adult member of the genus is that of 1803, when Rudolphi described *Fasciola macrostoma*. However, Rudolphi at this time did not recognise the generic significance of the species he described. It was not until Zeller (1874) had worked out the life history of the larval form described by Carus, showing that it developed into an adult identical with *Fasciola macrostoma* Rudolphi, that an adult fluke of the genus was recognised. Further

notes on the history of the genus have been given by Magath (1920) and need not be repeated here.

Distribution.

The species of the genus *Leucochloridium* are not confined to any special zone or climate, but, like other bird-fluke genera, are to be found in many parts of the world. Due to the migratory habit of the bird host this is to be expected, as has been pointed out by Nicoll (1923) for bird flukes in general. Lühe (1909) in his work gives only *L. macrostomum* (Rud.) from Germany. Nicoll records this same parasite from 17 species of British birds. He also records *L. turanicum* (Solowiow) and *L. insigne* (Looss) as being present.

Leucochloridium insigne was reported first by Looss (1899) from Egypt. *L. turanicum* was reported for the first time by Solowiow (1912) from Turkestan. In 1893 Monticelli described *L. cercatum* but did not know the host. Two species are listed by Viana (1924) from South America: *L. parcum* Travassos and *L. flavum* Travassos. Although the last named species was not described until 1922, Lutz (1921) had previously found it in birds and snails.

In America, a larval form from snails, *L. problematicum*, was described in 1920 by Magath. He points out that immature stages of *Leucochloridium* have been observed several times by malacologists when collecting snails. According to this author one of the earliest records of *Leucochloridium* in America is that reported by W. H. Dall (1892), mentioning that a fluke worm was found in *Succinea*, which he called *Leucochloridium americanum* Dall. This name was given without a description, and hence must be ignored.

Source of New Material.

In the course of the present study, flukes belonging to the genus *Leucochloridium* Carus were found parasitic in the intestine of the following birds:

Host	No. of specimens	Date	Locality
White-eyed Vireo (*Vireo griseus griseus*)	1	16. IX. 24	Mississippi
Baltimore Oriole (*Icterus galbula*)	3	15. VI. 25	Minnesota
Black and White Warbler (*Mniotilta varia*)	4	3. IX. 25	Minnesota
Sora Rail (*Porzana carolina*)	7	23. V. 26	Minnesota
Brown Creeper (*Certhia familiaris americana*)	7	8. X. 26	Minnesota

Genus *Leucochloridium* Carus.

That these flukes belong to the genus *Leucochloridium* is evident from the following characteristics.

Small distomes with two large powerful suckers about equal in size. Pharynx large, muscular, following immediately after oral sucker, and opening into a very short oesophagus (oesophagus not distinguishable in whole mounts). Intestinal crura extending to posterior end of body or very nearly so. Reproductive organs in posterior half of body with ovary in zone between testes; never appearing lobed, but varying from egg-shape to more or less spherical. Uterus with numerous eggs, filling most of intercoecal space, and almost

completely obliterating the view of ovary and testes. Genital opening at or near posterior end of body (terminal, dorsal, or ventral). Cirrus sac present. Laurer's canal present. Excretory pore dorsal near posterior end of body. Vitellaria extracoecal, continuous throughout greater part of body-length.

In each case these parasites proved to be previously undescribed species as described below.

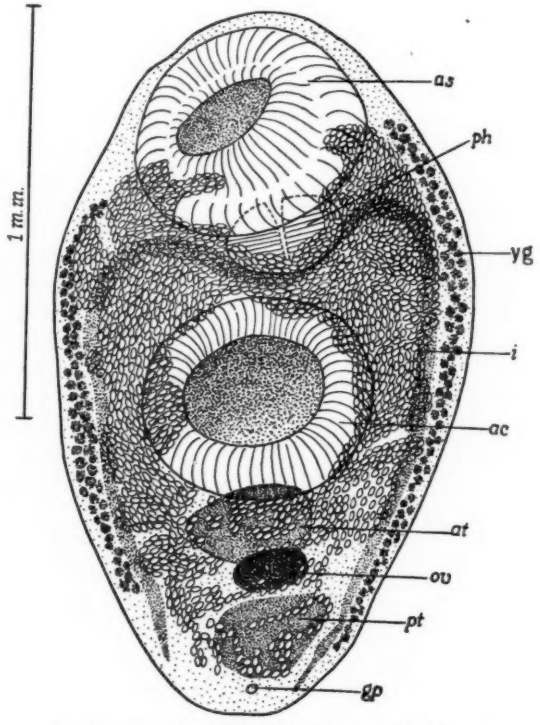


Fig. 1. *Leucochloridium certhiae* n. sp., ventral aspect.

ABBREVIATIONS USED IN FIGURES 1–5.

ac	acetabulum	*as*	anterior sucker	*at*	anterior testis	*c*	cirrus
cs	cirrus sac	*ed*	excretory duct	*ep*	excretory pore	*gp*	genital pore
i	intestinal crura	*ot*	ootype	*ov*	ovary	*ph*	pharynx
pt	posterior testis	*ut*	uterus	*vd*	vas deferens	*yg*	yoke glands

The line by the side of each drawing represents 1 mm.

DESCRIPTION OF NEW SPECIES.

Leucochloridium certhiae n. sp. (Fig. 1).

Length 1·6848 mm.; width 1·0206 mm.; measurements from compressed specimen. In specimen sectioned and not compressed, dorsal surface of body very strongly convex, ventral surface slightly convex. Maximum width of sectioned specimen, 0·6318 mm.; greatest thickness, 0·4617 mm. Oral sucker

terminal (slightly depressed ventrally in specimen illustrated), measuring 0·4536 by 0·5340 mm. Pharynx muscular, following immediately after oral sucker, diameter, 0·2242 mm., length, 0·1520 mm. Oesophagus very short, distinguishable only in cross-section. Acetabulum about equal in diameter to

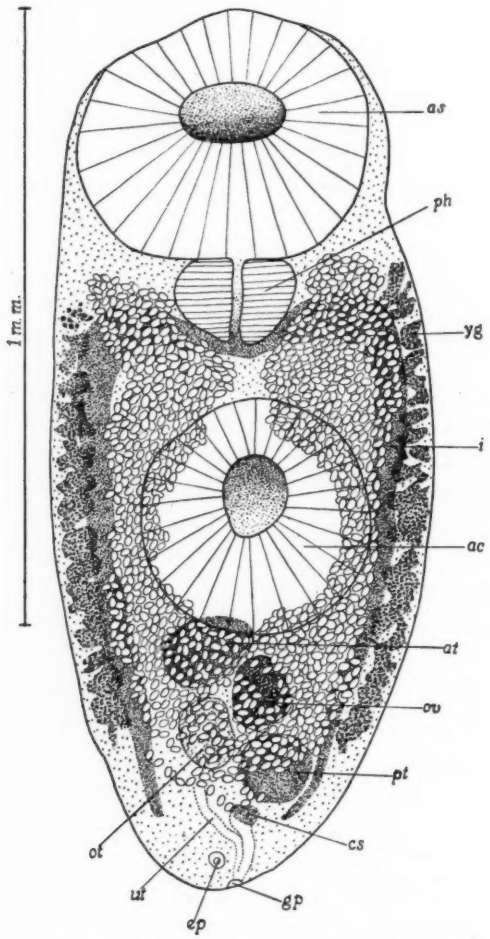


Fig. 2. *Leucochloridium mniotiltae* n. sp., ventral aspect.

oral sucker, measuring 0·4860 by 0·5340 mm. Genital pore median and terminal. Cirrus sac small, length 0·18 mm., diameter 0·07 mm. Cirrus small, extending into genital atrium to opening of genital pore. Testes large; anterior testis partly overlapped on anterior margin by acetabulum, posterior margin overlapping anterior margin of ovary. Posterior testis directly posterior to ovary

and adjacent to it, about equal in size to anterior testis, measuring 0·19 by 0·32 mm. In sectioned specimen anterior testis 0·2268 by 0·24 mm.; posterior, slightly larger measuring 0·2268 by 0·2560 mm. Vas efferens of anterior testis large and convoluted, acting as a seminal vesicle for this organ. Ovary small, egg-shaped, situated between testes, almost entirely to left of median line of body, measuring 0·12 by 0·20 mm. In sectioned specimen ovary almost spherical, measuring 0·12 mm. in diameter. Uterus filled with eggs, much convoluted, extending laterally on each side to vitellaria, anteriorly to oral sucker and ending posteriorly to right of median line, emptying into genital atrium. Eggs numerous, 0·0266 by 0·0190 mm.; light yellow to very dark brown, darkest coloured eggs in right side of body at posterior end. Vitellaria extra-coecal extending anteriorly to about one-third of the depth of anterior sucker, and posteriorly to about middle level of posterior testis.

Habitat: Cloaca of Brown Creeper (*Certhia familiaris americana*).

Leucochloridium mniotiltae n. sp. (Fig. 2).

Length 1·4134 mm.; width 0·6318 mm.; oral sucker 0·3888 by 0·4536 mm.; pharynx 0·1296 mm. long, 0·1944 mm. in diameter; oesophagus very short; intestinal crura widest at point of curvature, tapering gradually and ending slightly posterior to zone of posterior testis. Acetabulum 0·3888 mm. in diameter. Testes about equal in size, measuring 0·1292 by 0·1102 mm. Anterior testis overlapped ventrally on its anterior margin by ventral sucker, the greater part to right of median line, but not touching intestinal coecum. Posterior testis diagonally posterior to anterior testis, a little removed from median line and adjacent to inner margin of intestinal coecum. Ovary a little longer than wide, measuring 0·114 by 0·100 mm., occupying space between the two testes. Ootype present, about equal to ovary in size and situated to right of posterior testis. Uterus filled with eggs, occupying intercoecal space in area of reproductive organs, forward to anterior level of pharynx; area of pharynx and most of acetabulum free from eggs. Eggs 0·030 by 0·019 mm. Genital pore median and terminal. Cirrus sac small, 0·120 mm. long, 0·070 mm. in diameter. Vitellaria extra-coecal, extending from about level of anterior margin of pharynx to about equatorial level of posterior testis. Excretory pore dorsal, about 0·048 mm. from posterior end. Laurer's canal opening dorsally, about 0·016 mm. anterior to excretory pore.

Habitat: Large intestine of Black and White Warbler (*Mniotilta varia*).

Leucochloridium icteri n. sp. (Fig. 3).

Length 2 mm.; width 1·053 mm. in specimen slightly compressed. Oral sucker 0·405 by 0·4455 mm.; acetabulum 0·4212 by 0·4536 mm.; pharynx 0·1296 mm. long, 0·1782 mm. in diameter. Oesophagus very short; intestinal crura very distinct, extending lateral to vitellaria, then turning abruptly posteriorly to about equatorial level of posterior testis. Vitellaria very

pronounced, extra-coeeal, extending from near middle of zone of oral sucker to about equatorial level of ovary. Uterus very much convoluted, filled with eggs and occupying most of available space posterior to anterior sucker. Eggs 0·0251 by 0·0152 mm., light yellow to very dark brown, the dark brown, more mature eggs surrounding the reproductive organs. Testes almost spherical, about equal in diameter, measuring 0·17 mm.;

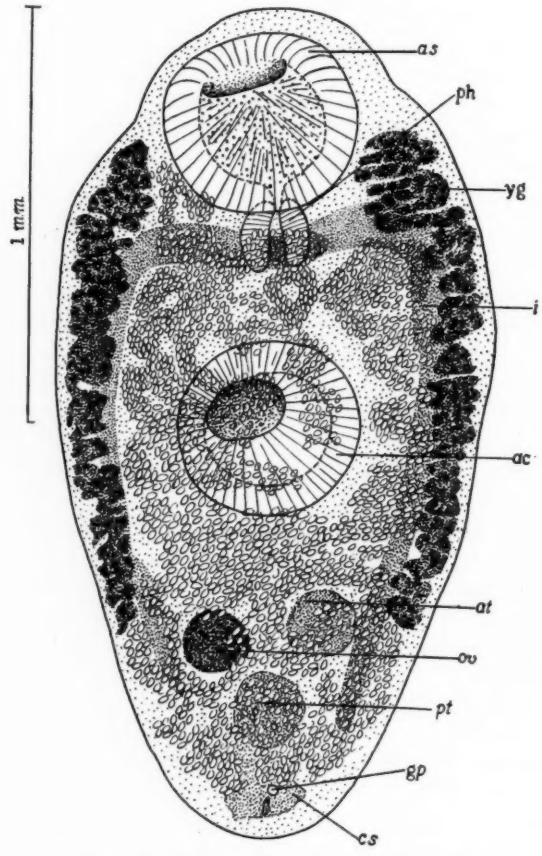


Fig. 3. *Leucochloridium icteri* n. sp., ventral aspect.

anterior testis to left of median line and separated one and one-fourth times its diameter from acetabulum; posterior testis located in median line, about equal distance from and at level of ends of crura, and separated by less than half its diameter from anterior testis and ovary. Ovary spherical, situated to left of median line and opposite the testicular interval, a little nearer the posterior than the anterior testis; the diameter is somewhat less than that

of either testis, measuring 0·1539 mm. In whole mounts when viewed from ventral side the testes almost completely obscured by dark-coloured eggs. The three reproductive organs form points of a triangle which approaches that of an equilateral triangle. Cirrus sac present, 0·196 mm. long, 0·065 mm. in diameter. Genital pore median and ventral, about 0·070 mm. from posterior end. Uterus entering the very short genital atrium on left side from cirrus sac. In other species studied from cross-sections the uterus enters on right side from cirrus sac. Excretory pore dorsal, about 0·028 mm. from posterior end; excretory canal extending anteriorly for 0·056 mm. before branching. Laurer's canal not recognisable. Many large nucleated cells surrounding vasa efferentia and vas deferens, probably prostatic in function.

Habitat: Rectum of Baltimore Oriole (*Icterus galbula*).

Leucochloridium vireonis n. sp. (Fig. 4).

Length 2·5 mm.; width 0·95 mm.; body flat, width nearly uniform throughout, maximum breadth at posterior region of pharynx. Oral sucker somewhat broader than long, measuring 0·47 by 0·53 mm. Pharynx muscular, connected directly to oral sucker, length 0·1701 mm., diameter 0·2106 mm. Oesophagus very short or absent. Intestinal crura extending diagonally anteriorly on leaving oesophagus for about half the length of pharynx, then passing posteriorly to equatorial level of posterior testis; diameter of crura increasing at posterior extremities. Acetabulum slightly anterior to middle of body, diameter 0·57 mm. Anterior testis about equal distance from acetabulum and posterior end of body left of median line; length 0·2268 mm., width 0·1944 mm. Posterior testis 0·3078 by 0·2592 mm., situated between ends of intestinal crura, about one-third its diameter from posterior end of body and nearly median in position. Ovary to right of median line with its anterior margin at about equatorial level of anterior testis; almost spherical, about equal in size to anterior testis; measuring 0·2368 by 0·1822 mm. Uterus filled with eggs occupying the greater part of the body space posterior to anterior sucker, and overlapping intestinal crura and vitellaria in a few places. Eggs numerous, measuring 0·0247 by 0·0152 mm., light brown to dark brown in colour, the dark brown, more mature eggs posterior to ovarian zone and to left of testicular area. Vitellaria extra-coecal, extending from a point slightly anterior to posterior margin of oral sucker, to middle of ovarian zone; few of vitellarian follicles extending to inner margin of intestinal crura. Cirrus sac large, posterior and ventral to posterior testis.

Habitat: Large intestine of White-eyed Vireo (*Vireo griseus griseus*).

Leucochloridium sorae n. sp. (Fig. 5).

Length 2·5596 mm.; width 0·8748 mm., measurement taken at acetabulum; ventral surface of body flat, dorsal surface convex, tapering at posterior end, otherwise about the same breadth throughout. Suckers about equal in

diameter; anterior sucker 0·534 by 0·5994 mm.; acetabulum 0·5832 by 0·5994 mm. situated anterior to middle of body. Pharynx 0·1701 by 0·2106 mm. Anterior testis oval, 0·150 by 0·225 mm., on right side of body, and about twice its length (0·30 mm.) from acetabulum. Posterior testis a little smaller, 0·112 by 0·17 mm., on left side of body, and removed from zone of anterior testis

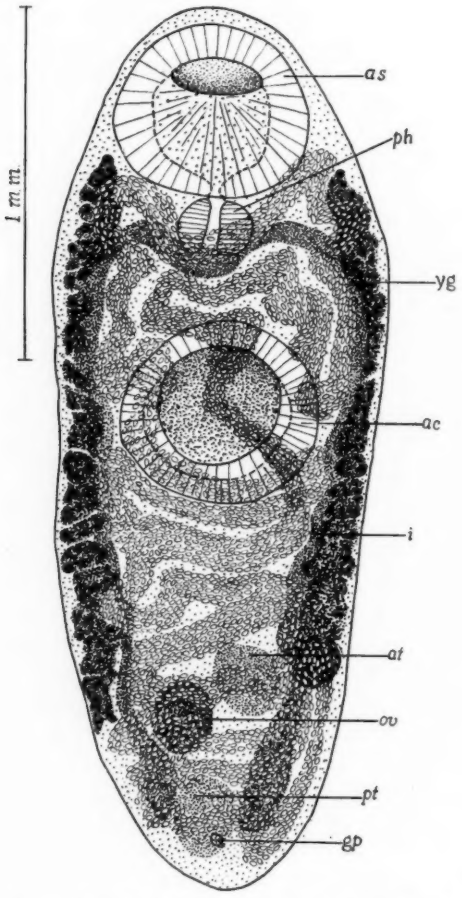


Fig. 4. *Leucochloridium vireonis* n. sp., ventral aspect.

for a distance greater than its length. Ovary 0·104 by 0·150 mm., anterior to posterior testis; fields of these two organs in contact and may overlap slightly. Uterus confined to intercoecal area. Cirrus sac large, length 0·176 mm., diameter 0·110 mm. Anterior part of vas deferens enlarged, forming seminal vesicle. Genital pore dorsal and median, near posterior end of body. Cirrus

protruding from genital atrium, measuring 0·128 by 0·060 mm. Uterus entering genital atrium on right side from cirrus sac. Eggs yellowish-brown, 0·0226 by 0·0152 mm. Excretory pore dorsal, 0·064 mm. from posterior end.

Habitat: Intestine of Sora Rail (*Porzana carolina*).

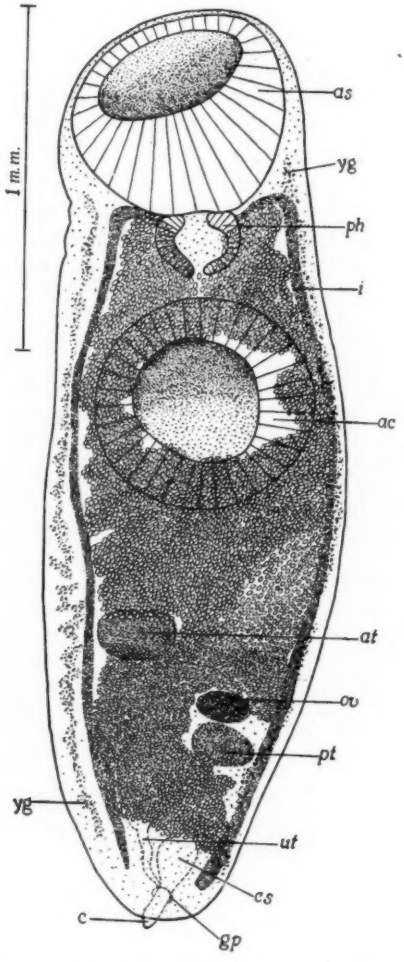


Fig. 5. *Leucochloridium sorae* n. sp., ventral aspect.

Table of Measurements.

	Length	Width	Oral Sucker	Ventral Sucker	Pharynx	Ant. Testis	Post. Testis	Ovary	Cirrus Sac	Eggs	Host
L. macrostomum			·20						·15	·023	*Rallus aquaticus*
	1·8	0·90	·20	·20	·16	·20	·22	·20	·16	·016	
L. cercatum									·13		Unknown
	4·0	1·20	·60	·72	·22	·26	·26	·24	·34		
L. insigne									·13	·029	*Fulica atra*
	3·0	1·35	·73	·69	·30	·22	·20	·13	·33	·015	
L. turanicum				·69	·09				·1165	·023	*Totanus gearcola*
	3·5	1·4	·69	·79	·18	·3	·4	·233	·319	·0093	
L. flavum					·34	·60	·60	lobed	large	·023	*Gallinula galeata*
	5·5	2·5	1·30	1·30	·41	·40	·50			·017	
L. parcum			·50		·12	·26	·26	·10	large	·028	*Ostinops decumanus*
	2·10	1·00	·50	·53	·19	·20	·17	·13	·31	·017	
L. certhiae			·45	·48	·15	·24	·25	·12	·07	·026	*Certhia familiaris*
	1·68	1·02	·53	·53	·22	·22	·22	·20	·18	·019	*americana*
L. mniotiltae			·38	·38	·129	·129	·129	·114	·07	·030	*Mniotilta varia*
	1·41	0·63	·45	·38	·194	·110	·110	·100	·12	·019	
L. icteri			·40	·42	·12	·17	·17	·153	·065	·025	*Icterus galbula*
	2·00	1·05	·44	·45	·17	·17	·17	·153	·196	·015	
L. vireonis			·47	·57	·17	·22	·30	·22		·024	*Vireo griseus griseus*
	2·5	0·95	·53	·57	·21	·19	·25	·18		·015	
L. sorae			·53	·583	·17	·15	·11	·10	·11	·022	*Porzana carolina*
	2·5	0·87	·59	·599	·21	·22	·17	·15	·17	·015	
(Larval form)									·07		*Succinea retusa*
L. problematicum	2·2	0·85	·39	·34	·15	·074	·061	·052	·16		*Planorbis trivolvis*

All measurements in mm.

KEY TO THE KNOWN SPECIES OF *LEUCOCHLORIDIUM*.

1. Ovary and testes lobed; over 5 mm. in length . . . . . *L. flavum* Trav.
   Ovary and testes oval or spherical; less than 5 mm. in length 2
2. Vitellaria extending well beyond posterior testis 3
   Vitellaria at most extending to posterior testis but never beyond it . . . 6
3. Ovary posterior to testicular zones . . . . . *L. cercatus* (Mont.).
   Ovary anterior to posterior testicular zone 4
4. Acetabulum in centre of body; vitellaria extending only to pharynx *L. insigne* (Looss).
   Acetabulum anterior to centre of body; vitellaria extending beyond pharynx . 5
5. Genital pore ventral; ovary and posterior testis on right side of body; eggs more than twice as long as broad . . . . . *L. turanicum* (Sol.).
   Genital pore dorsal; ovary and posterior testis on left side of body; eggs less than twice as long as broad . . . . . *L. sorae* n. sp.
6. Vitellaria not extending anteriorly beyond zone of ventral sucker *L. parcum* Trav.
   Vitellaria extending anteriorly well beyond zone of ventral sucker 7
7. Anterior testis adjacent to or partly overlapped by acetabulum 8
   Anterior testis removed from acetabulum 9
8. Testes large, about twice diameter of ovary . . . . . *L. certhiae* n. sp.
   Testes small, diameter about equal to ovary . . . . *L. mniotiltae* n. sp.
9. Anterior testis removed for a distance equal to its diameter, or less, from acetabulum; ovary to left of median line of body . . . . . *L. macrostomum* (Rud.)
   Anterior testis removed a distance greater than its diameter from acetabulum; ovary to right of median line of body 10
10. Distance separating posterior testis from tip of body about equal to distance separating anterior testis from acetabulum . . . . . *L. icteri* n. sp.
    Distance separating posterior testis from tip of body much less than distance separating anterior testis from acetabulum . . . . . *L. vireonis* n. sp.

DISCUSSION.

In the preparation of this paper, no types of previously described species were studied, but descriptions and illustrations were carefully checked and compared with the species described here.

In the case of *L. parcum* Trav. no illustrations were given and only a short description. If the description as to the extension of the vitellaria is accurate, none of the American species described here will agree with it. However, if the vitellaria should extend forward beyond the posterior margin of anterior sucker, this species would be somewhat similar to *L. icteri* n. sp. These two species, *L. parcum* and *L. icteri*, have many points in common, such as size of body and organs, and being from related bird genera.

In *L. turanicum* (Sol.) the illustrations show the ovary and posterior testis on the right side of body and the genital openings on the ventral side. If the order were reversed the arrangement of the organs would be similar to that of *L. sorae* n. sp. However, there are other characters in which these two species differ, body length and size of eggs for example.

L. certhiae n. sp., *L. mniotiltae* n. sp. and *L. vireonis* n. sp. are distinctly different from any of the previously described species.

In the key to species *L. problematicum* Magath is omitted on account of it being a larval form. This species, however, is included in the table of measurements with the other described species.

The types of the new species are to be deposited in the United States National Museum, Washington, D.C.

SUMMARY.

Adult trematodes belonging to the genus *Leucochloridium* Carus are here reported for the first time from North America. These flukes are intestinal parasites of birds and proved to be previously undescribed species.

In the present paper a key separating the species of the genus is given, and the following are described and figured as new species:

Leucochloridium certhiae n. sp. from *Certhia familiaris americana;*
Leucochloridium mniotiltae n. sp. from *Mniotilta varia;*
Leucochloridium icteri n. sp. from *Icterus galbula;*
Leucochloridium vireonis n. sp. from *Vireo griseus griseus;*
Leucochloridium sorae n. sp. from *Porzana carolina.*

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